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THE KANSAS UNIVERSITY SCIENCE BULLETIN

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The attention of learned societies and other institutions which exchange scientific publications with the University of Kansas is called to the list of publications of this University on the third page of the cover of this issue.

Those marked "Supply exhausted" can not be furnished at all; those marked "Supply small" can not be furnished separately; those marked "Supply large" will gladly be furnished to any of our exchanges who may need them to complete their files.

Back numbers of the *Kansas University Quarterly* and *Geological Survey*, as far as possible, will be sent to those of our newer correspondents who are able and willing to reciprocate.

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THE KANSAS UNIVERSITY SCIENCE BULLETIN.

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CONTENTS:

NOTE ON A PROBLEM IN THE THEORY OF ALGEBRAIC MANIFOLDS,
S. Lefschetz.

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Note on a Problem in the Theory of Algebraic Manifolds.

BY S. LEFSCHETZ.

1. In a recent paper[1] I have solved the following problem: Let D_1, D_2, ... D_s, be algebraic manifolds belonging to an irreducible algebraic r-dimensional variety, of which the hypersurfaces C_1, C_2, ... C_ρ, form a base. If

$$\lambda_j D_j = \Sigma \lambda_i^j C_i ,$$

to obtain the genus of D_1, D_2, ... D_s, complete intersection of the D's, in terms of the genera of the C's and of their mutual intersections. It was shown there that a formula answering the purpose exists, independent from the signs of the integers λ, and this formula was explicitly given (formula 15). This was finally applied to loci of spaces, or generalized scrolls, and to complete intersections in ordinary r-space.

The solution obtained was based upon Severi's formula for the genus of a manifold sum of two others[2]. However, the fundamental formula was derived only by an indirect method. It is the main object of the present note to show how the problem in question can be solved by a *complete inversion* of Severi's formula, inversion which in itself is not without interest. We will make use throughout of the notion of *virtual manifolds* considered at length in the note already referred to. With this notion the problem is purely one of algebra, and consists in the extension of a certain algebraic functional operation. The abstract problem underlying the symbolism used is considered in §§ 7, 8. The interesting result is obtained that the most general addition for-

1. The arithmetic genus of an algebraic manifold immersed in another. Annals of Mathematics, 1916. Vol. 17, p. 197.

2. Fundamenti per la geometria sulle varieta algebriche. Rendiconti del Circolo Mathematico di Palermo, 1909, p. 42.

mula analogous to Severi's is given by $C=A+B$; $\{(\alpha A+\beta)(\alpha B+\beta)\} = \{\alpha C+\beta\}$, which for $\alpha=\beta=1$ yields Severi's.

2. The symbols used will be practically those of my previous paper. It will be convenient to recall them briefly.

The genus of a manifold M will be designated by $[M]$. Let $A, B, \ldots . C$, be hypersurfaces of the fundamental irreducible algebraic variety V_r, $f(A, B, \ldots . C)$ a power series proceeding according to positive powers of the symbols $A, B, C \ldots . .$ Then

(a) We will denote by $[f(A, B, \ldots . C)]$ the result obtained when the constant term is left unchanged; the term in $A^a B^b \ldots . C^c$ is replaced by $[A^a B^b \ldots . C^c]$ if its degree is $\leq r$, and by zero if it exceeds r.

(b) Let $F(x) = f(x, x, \ldots . x) = \sum_i a^i x^i$. We will write

$$\{f(A, B, \ldots . C)\} = [f(A, B, \ldots . C)] + (-1)^{r-1} \sum_{i=0}^{r-1} (-1)^i a^i.$$

This last definition is not identical with that of my paper, but is equivalent to it, as shown at the end of the introduction to it.

3. Let $D = C_1 + C_2$. Then[3]

$$\{1+D\} = \{(1+C_1)(1+C_2)\}. \tag{1}$$

The following generalizations of (1) have been obtained:

(a) If $D = \sum_{i=1}^{n} C_i$, $M = D_1 D_2 \ldots . D_h$, then

$$\{M(1+D)\} = \{M \prod_i (1+C_i)\}. \tag{2}$$

(b) If $D = C_1 - C_2$, $\{M(1+D)\} =$

$$\left\{M \frac{1+C_1}{1+C_2}\right\} \equiv \{M(1+C_1)(1+C_2)^{-1}\}, \tag{3}$$

where at the right the quotient is to be replaced by its expansion in power series.

From these formulas follows that if $D = \sum \lambda_i C_i$, where the λ's are any integers, then

$$\{M(1+D)\} = \{M \prod_i (1+C_i)^{\lambda_i}\}. \tag{4}$$

4. The formula to be proved will follow readily from the extension of (4) to the case where the λ's are any rational numbers. Suppose first that $\lambda D = C$, where λ is a positive integer. Then I propose to show that

$$\{M(1+D)\} = \{M(1+C)^{\frac{1}{\lambda}}\}, \tag{5}$$

3. The arithmetic genus . . . formula 1. This is slightly more general than the formula originally given by Severi.

whatever the manifold M. Proceed by induction. The formula is easily verified when

$$M = M_s = A_1 A_2 \ldots . A_s , \quad s > r .$$

Grant that it holds for $s > s$, I say that it is also true for $s = s_0$. For $\lambda M_{s_0} D = M_{s_0} C$;

$$\therefore \ \{ M_{s_0} (1+D)^{\lambda} \} = \{ M_{s_0} (1+C) \} . \tag{6}$$

But $M_{s_0} D^k = M_{s_0} D^{k-1} . D$.

Hence from the assumptions made follows:

$$\{ M_{s_0} D^{k-1} (1+D) \} = \{ M_{s_0} D^{k-1} (1+C)^{\frac{1}{\lambda}} \} ; k > 1$$

$$\therefore \ \{ M_{s_0} D^k \} = \{ M_{s_0} D^{k-1} (1+C)^{\frac{1}{\lambda}} - 1 \} .$$

This remains true when M_{s_0} is replaced by $M_{s_0} C^h$.

$$\therefore \ \{ M_{s_0} D^k \} = \{ M_{s_0} ((1+C)^{\frac{1}{\lambda}} - 1)^k \} , \ k > 1 . \tag{7}$$

From (6) follows

$$\{ M_{s_0} \sum_{k=0}^{\lambda} \binom{\lambda}{k} D^k \} = \{ M_{s_0} (1+C) \} .$$

This together with (7) gives finally

$$\{ M_{s_0} (1+C) \} =$$

$$\{ M_{s_0} \sum_{k=0}^{\lambda} \binom{\lambda}{k} D^k ((1+C)^{\frac{1}{\lambda}} - 1)^k + \lambda (1+D) - \lambda (1+C)^{\frac{1}{\lambda}} \} =$$

$$\{ M_{s_0} (((1+C)^{\frac{1}{\lambda}} - 1 + 1)^{\lambda} + \lambda (1+D) - \lambda (1+C)^{\frac{1}{\lambda}}) \} .$$

$$\therefore \ \{ M_{s_0} (1+D) - M_{s_0} (1+C)^{\frac{1}{\lambda}} \} = 0 ,$$

as was to be proved.

5. It remains to be shown that if $\lambda D = C_1 - C_2$, then

$$\{ M (1+D) \} = \{ M (1+C_1)^{\frac{1}{\lambda}} (1+C_2)^{-\frac{1}{\lambda}} \} . \tag{8}$$

If we set $\lambda D = D' = C_1 - C_2$, we have by (5)

$$\{ M (1+D) \} = \{ M (1+D')^{\frac{1}{\lambda}} \} . \tag{9}$$

Also,

$$\{ M(1+D') \} = \{ M(1+C_1)\,(1+C_2)^{-1} \} ;$$
$$\therefore \{ MD' \} = \{ M\,((1+C_1)\,(1+C_2)^{-1} - 1) \} .$$

Hence finally by (9)

$$\{ M(1+D) \} = \{ M(1+D')^{\frac{1}{\lambda}} \} = \{ M \sum_{k=0}^{\nu} \binom{\frac{1}{\lambda}}{k} D'^{k} \} =$$
$$\{ M \sum_{k=0}^{\nu} \binom{\frac{1}{\lambda}}{k} D^{k} ((1+C_1)\,(1+C_2)^{-1} - 1)^{k} \} =$$
$$\{ M\,((1+C_1)\,(1+C_2)^{-1})^{\frac{1}{\lambda}} \} =$$
$$\{ M\,(1+C_1)^{\frac{1}{\lambda}}\,(1+C_2)^{-\frac{1}{\lambda}} \}$$

as was to be proved.

6. From (5) and (8) follows that if $\lambda D = C_1 \pm C_2$, then

$$\{ f(D, A, B, \ldots C) \} = \{ f(\,(1+C_1)^{\frac{1}{\lambda}}\,(1+C_2)^{\pm\frac{1}{\lambda}} - 1, A, B, \ldots C) \}$$

f being as usual a power series.

Let $\lambda D = \sum_{i=1}^{\nu} \lambda_i C_i$. We may set $\lambda_1 C = \pm C_1'$, $\sum_{i=2}^{\nu} \lambda_i C_i = D_1$, according as λ_1 is positive or negative. Then $\lambda D = D_1 \pm C_1' = D_1 + \lambda_1 C_1$.

$$\therefore \{ f(D, A, \ldots B) \} = \{ f(\,(1+D_1)^{\frac{1}{\lambda}}\,(1+C_1')^{\pm\frac{1}{\lambda}} - 1, A, \ldots . B) \} ,$$

and as $\{ \Phi(C_1', A, \ldots B) \} = \{ \Phi(\,(1+C_1)^{\pm\frac{\lambda_1}{\lambda}} - 1, A, \ldots B) \}$ it follows

$$\{ f(D, A, \ldots B) \} = \{ f(\,(1+C_1)^{\frac{\lambda_1}{\lambda}}\,(1+D_1)^{\frac{1}{\lambda}} - 1, A, \ldots B) \} .$$

and this by a repeated application leads to the following very general formula

$$\{ f(D, A, \ldots B) \} = \{ f(\,(\prod_i (1+C_i)^{\frac{\lambda_i}{\lambda}} - 1, A, \ldots B) \} . \qquad (10)$$

From this follows immediately the fundamental formula already referred to; for if

$$\lambda_j D_j = \sum_{i=1}^{\rho} \lambda_i^j C_i\,, \quad (j = 1, 2, \ldots . s)$$

we have

$$\{ D_1 D_2 \ldots D_s \} = \{ \prod_{i=1}^{s} (\prod_{i=1}^{\rho} (1 + C_i)^{\frac{\lambda_i^j}{\lambda_j}} - 1) \}$$

which is (15) of the paper already quoted, but in a different form. If at the right we wished, as there done, to have the sign [ ] in place of { }, we would have to add an integer N. To obtain its value in terms of the λ's we remark that according to §2, N can be expressed as a polynomial in the ratios $\frac{\lambda_i^j}{\lambda_j}$. We may therefore make $\lambda_j = 1$, for all values of j, and find N somewhat as done *loc. cit.* §4. This calculation will be omitted here.

7. The theory underlying the preceding discussion may be abstractly stated thus: Let $A_1, A_2, \ldots .$ be a finite or infinite set of magnitudes such that the sum $\Sigma \lambda_i A_i$, where the λ's are arbitrary integers, defines a quantity of the set. This quantity is supposed to remain unchanged when the A's are arbitrarily permuted together with their coefficients; that is, the commutative law of addition holds. Furthermore, the associative law is also assumed. Besides the above quantities, we consider their products in finite number $M = A_1 A_2 \ldots . A_s$. If r is an arbitrary but fixed positive integer, the number $r - s = d$ is called the dimensionality of M. We then define a function of M called its genus $[M]$ by the following properties:

(a) $[M]$ is uniquely determined when M is known.

(b) With the notations defined previously,

$$B = A_1 + A_2\,; \quad \{ M\ (1 + B) \} = \{ M\ (1 + A_1)\ (1 + A_2) \}\,.$$

These two conditions suffice to make certain that if the numbers $[A_1^i\ A_2^j \ldots . A_s^k]$ are known we can obtain $[D_1\ D_2 \ldots . D_h]$, where $\lambda_j D_j = \sum_{i=1}^{s} \lambda_i^j A_i$. In particular if there is a base in the

usual sense for the set of the A's, we can obtain all the genera $[M]$ as linear functions of the base genera.

Starting from the above properties, all the formulas obtained could be derived without having recourse to virtual manifolds, as becomes necessary with the more specialized geometric theory already discussed.

In this connection three questions present themselves:

(a) Is it possible to obtain other systems than that of algebraic manifolds contained in a given variety, having the properties outlined above?

(b) Given a base set $C_1, C_2, \ldots C_\rho$, the system defined by all the C's such that $\lambda C = \Sigma \lambda_i C_i$, and a system of integral values for the genera $[C_1^i C_2^j \ldots . C_\rho^k]$, is there an irreducible algebraic variety such that its hypersurfaces are in one-one correspondence with the system so defined?

(c) Are there laws other than the one corresponding to Severi's addition formula, and yielding results of interest?

Very little may be said at present in regard to (a) and (b), but more concerning (c), which is what we proceed to do.

8. The question must first be stated more definitely. It is proposed to consider addition formulas of the type

$$\{ Mf(C_1+C_2) \} = \{ M\phi(C_1, C_2) \},$$

where f, ϕ, are polynomials. The simplest of this type is perhaps the following:

$$\{ Mf(C_1+C_2) \} = \{ Mf(C_1)f(C_2) \} \qquad (11)$$

which can be easily shown to lead to the result

$$\lambda D = \Sigma \lambda_i C_i\,; \quad \{ f(D) \} = \left\{ \overline{\prod_i} f(C_i)^{\frac{\lambda_i}{\lambda}} \right\}.$$

The difficulty with laws of this general type lies in the fact that unless f is linear, $[\overline{C_1+C_2}]$ can not be obtained readily in terms of the genera $[C_1^i C_2^k]$. Restricting ourselves, therefore, to the case where $[C_1+C_2]$ can be obtained directly in terms of these genera, we propose to show that: *Addition formulas yield-*

ing directly $[M(C_1+C_2)]$ *in terms of the genera* $[MC_1^i\ C_2^k]$, *are all of the type* (11), *f being of the first degree.*

Suppose that $\{M(A+B)\} = \{Mf(A,B)\}$. Clearly $f(A,B)$ must be symmetric in A, B, this being a direct consequence of the commutative law. Furthermore,

$$\{M(A+B+C)\} = \{M\phi(A,B,C)\},$$

where ϕ is a polynominal which, for the same reason, must be symmetric in A, B, C. Let

$$f(A,B) = \Sigma \alpha_{ik} A^i B^k,$$

and denote by μ the highest power at which either A or B occurs in f. Then, since

$$\phi(A,B,C) = \Sigma \alpha_{ik} A^i (\Sigma \alpha_{mn} B^m C^n)^k,$$

it is easily seen that in ϕ, A will be found at most to the power μ, while if r is sufficiently high, there will be a term containing C with the exponent μ^2.

$$\therefore\ \mu^2 = \mu,\ \mu = 1.$$

Thus the polynomial f must be of the first degree. As it is also symmetric, we have

$$f(A,B) = a(A+b)(B+b) + c,$$

where a, b, c are certain constants still in part to be determined. From this follows

$$\phi(A,B,C) = a(A+b)(a(B+b)(C+b)+c)+c,$$

and the condition of symmetry will be fulfilled only if $b = -c$, when $f(A,B) = a(A+b)(B+b) - b$.

$$\therefore\ \{a(A+B+b)\} = \{a(A+b).a(B+b)\}.$$

If we set $a = \alpha$, $ab = \beta$, we have

$$C = A+B;\ \{M(\alpha C+\beta)\} = \{M(\alpha A+\beta)(\alpha B+\beta)\}$$

as was to be proved.

LAWRENCE, KAN., May 30, 1916

□

THE

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CONTENTS:

AN INVESTIGATION OF THE CONDITION OF THE DRINKING WATER ON TRAINS OPERATING IN OR THROUGH KANSAS, . . *N. P. Sherwood*

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An Investigation of the Condition of the Drinking Water on Trains Operating In or Through Kansas.*

BY N. P. SHERWOOD.

(From the Department of Bacteriology of the University of Kansas, Lawrence.)

AT the request of Dr. S. J. Crumbine, secretary of the Kansas State Board of Health, an examination of the drinking water on trains that operate in or through Kansas was begun on May 23, 1912, at the Kansas City union station. A similar examination was made at Wichita on May 29 and 30. Fifty-four samples were taken at Kansas City from trains on the Frisco, Santa Fe, M. K. & T., Missouri Pacific, Burlington and Union Pacific railroads. The samples were plated on plain agar immediately after collection, and a set of six fermentation tubes containing Jackson's lactose-bile[1] were also inoculated. Five of these tubes were each inoculated with 1 cc. of the sample of water, and 10 cc. of the sample was planted into one large fermentation tube. This was done in order to determine approximately how many, if any, *B. coli* were present. These plates and tubes were then incubated for twenty-four hours at 37° C., and counts made.

There are present, even in good drinking-water, a few harmless bacteria, the number varying largely with the source of supply. These harmless water bacteria, however, thrive best at room temperature, and are, as a rule, inhibited when incubated at 37° C., or body temperature.

Mathews,[2] in 1893, showed that in streams presumably exposed to pollution the count at 37° C. was in the neighborhood of 100 per cc., while for unpolluted water from wells, springs and taps the count was under 30 per cc. Gage[3] has shown that for clean,

*Read before the Kansas State Board of Health, June 6, 1912.

1. Jour. Infec. Dis., March, 1906.

2. Technology Quarterly VI, 241.

3. A Study of the Number of Bacteria Developing at Different Temperatures, and of the Relation between such Numbers with Reference to their Significance in the Interpretation of Water Analysis.—Biological Studies by the Pupils of William Thompson Sedgwick, Boston, 1906.

unpolluted water the number of organisms developing at 37° C. is quite small, being, as a rule, under 50 per cc., while for polluted water the count is high. Too much stress must not be laid upon the count alone, yet one can get some idea as to whether the water is at least clean or dirty by the number of bacteria developing at the body temperature.

For a long time bacteriologists considered that the character of a water could be determined by the number of bacteria developing on agar and gelatine. Eventually bacteriologists realized that the quality or kind of bacteria present, as well as the quantity, is of prime importance. The customary qualitative test made on any sample of water is one to determine whether *Bacillus coli* is present, and if so, in what quantity. A test is made for this particular intestinal organism, because if it is present it is easily found and identified, and since it is a normal inhabitant of the colon or large intestines of warm-blooded animals, it indicates, when present in sufficient quantity, *i. e.*, 1 per cc. or more, pollution with animal waste.

In England, where the sewage is much more concentrated than in America, sewage streptococci are looked for in water examination instead of *B. coli*. The English argue, with reason, that the sewage streptococci are more delicate than *Bacillus coli*, and hence their presence in a water is a certain indication of *recent* sewage pollution. In these examinations streptococci were looked for as well as *Bacillus coli*. Daniel D. Jackson, in the *Journal of Infectious Diseases*, March, 1907, defines *Bacillus coli* as a Gram negative organism capable of fermenting both dextrose and lactose, with the production of carbon dioxide and hydrogen, which does not liquefy gelatin in fourteen days and which is morphologically a rod.

It is a well-known fact that ordinary ox bile has an inhibitive action on the majority of bacteria outside of those belonging to the typhoid and colon groups. Also that comparatively few organisms outside the colon group ferment lactose. Hence lactose bile—ordinary ox bile to which has been added one per cent peptone and one per cent lactose—is a medium made use of in examining a water for *Bacillus coli*. Measured quantities of water are added to fermentation tubes containing lactose bile; and if *Bacillus coli* is present the lactose will be fermented and carbon dioxide and hydrogen will be given off. When gas showed up in any of the fermentation tubes, confirmatory tests were made to see whether it was *B. coli* or some other organism that was causing

the fermentation. As a rule, in about 90 per cent of the cases *B. coli* will be found to be the active agent. *Streptococcus pyogenes* was finally identified by its characteristic growth on blood agar. As a general thing, only one sample was taken from each train, and that was collected from the chair car next to the smoker.

The results of the work done at Kansas City, Mo., and at Wichita, Kan., are shown by the tables on pages 16, 17, and 18.

Report on examination of water taken from coolers of trains arriving and departing from Kansas City, Mo., and Wichita, Kan.

FROM KANSAS CITY, MO., MAY 23 AND 24, 1912.

Sample No.	Train No.	Name of Railroad.	Time.	Train arriving or departing.	Date, 1912.	Bacteria per cc. on agar at 37° C, 24 hours.	Gas in lactose bile.						Approximate number of *B. coli* per 10 cc.	Remarks.
							1 cc.					10 cc.	Confirmatory test.	
							1	2	3	4	5			
1	102	Frisco	5:00 p. m.	A	5–23	100	–	–	–	–	–	–	None.	None.	
2	125	Frisco	5:05 p. m.	D	5–23	150	–	–	–	–	–	–		None.	
3	10	A. T. & S. F.	5:05 p. m.	D	5–23	650	–	–	–	–	–	–		None.	
4	24	M. K. & T.	5:05 p. m.	A	5–23	300	–	–	–	–	–	–		None.	
5	12	Rock Island	5:10 p. m.	A	5–23	1,400	–	–	–	–	–	–			
6	29	M. K. & T.	5:30 p. m.	D	5–23	250	–	–	–	–	–	–			
7	137	Frisco	5:35 p. m.	D	5–23	850	–	–	–	–	–	–			
8	1	Mo. Pacific	6:00 p. m.	D	5–23	1,200	+	–	–	–	–	+	+	2	
9	39	Rock Island	6:10 p. m.	D	5–23	200	–	–	–	–	–	–			
10	106	Mo. Pacific	6:15 p. m.	A	5–23	400	–	–	–	–	–	+	+	1	
11	105	Frisco	6:15 p. m.	D	5–23	750	+	–	+	–	–	+	+	4	
12	119	Mo. Pacific	6:20 p. m.	D	5–25	800	+	+	–	–	–	+	+	4	
13	43	Burlington	6:35 p. m.	D	5–23	590	–	–	–	–	–	–	–	None.	
14	111	Frisco	9:10 p. m.	D	5–23	500	–	–	–	–	–	+	+	1	
15	11	A. T. & S. F.	9:25 p. m.	D	5–23	650	–	+	–	–	–	+	+	2	
16	36	Rock Island	9:45 p. m.	A	5–23	250	–	–	–	–	–	–		None.	
17	103	Mo. Pacific	9:50 p. m.	D	5–23	300	–	–	–	–	–	–		None.	
18	17	A. T. & S. F.	10:00 p. m.	D	5–23	800	–	–	–	–	–	–		None.	
19	21	M. K. & T.	10:00 p. m.	D	5–23	950	–	+	+	–	–	+	+	4	
20	112	Mo. Pacific	10:20 p. m.	A	5–23	150	–	–	–	–	–	–		None.	
21	203	A. T. & S. F.	10:45 p. m.	D	5–23	1,100	–	–	–	–	–	–		None.	
22	1	Rock Island	11:15 p. m.	D	5–23	800	+	+	–	+	–	+	+	6	
23	23	Rock Island	11:30 p. m.	D	5–23	250	–	–	–	–	–	–		None.	
24	25	M. K. & T.	2:20 a. m.	D	5–24	1,500	–	–	–	–	–	+	+	1	
25	210	Mo. Pacific	2:00 a. m.	D	5–24	40	–	–	–	–	–	–	–	None.	
26	23	Rock Island	6:15 a. m.	A	5–24	250	–	–	–	–	–	+	+	1	
27	8	A. T. & S. F.	6:30 a. m.	A	5–24	700	–	–	–	–	–	–	–	None.	*Streptococcus pyogenes* present.
28	18	A. T. & S. F.	7:00 a. m.	A	5–24	550	–	–	–	–	–	–		None.	
29	12	A. T. & S. F.	7:10 a. m.	A	5–24	620	–	–	–	–	–	–		None.	
30	104	Frisco	7:00 a. m.	A	5–24	200	–	–	–	–	–	–		None.	
31	22	M. K. & T.	7:20 a. m.	A	5–24	300	–	–	–	–	–	–		None.	
32	35	Rock Island	7:55 a. m.	D	5–24	1,000	–	–	–	–	–	+	–	None.	*B. mycoides.*
33	113	A. T. & S. F.	8:01 a. m.	D	5–24	600	–	–	–	–	–	–		None.	
34	107	Union Pacific	8:05 a. m.	D	5–24	1,100	–	–	–	–	–	–	–	1	*Streptococcus pyogenes* present.
35	111	Mo. Pacific	8:15 a. m.	D	5–24	1,000	+	–	+	+	+	+	+	8	
36	127	Frisco	8:30 a. m.	D	5–24	30	–	–	–	–	–	+	Bubble.	None.	*B. mycoides.*

FROM KANSAS CITY, MO., MAY 23 AND 24, 1912—CONCLUDED.

Sample No.	Train No.	Name of Railroad.	Time.	Train arriving or departing.	Date, 1912.	Bacteria per cc. on agar at 37° C, 24 hours.	Gas in lactose bile.						Approximate number of *B. coli* per 10 cc.	Remarks.
							1 cc.					10 cc.	Confirmatory test.	
							1	2	5	4	5			
37	201	A. T. & S. F.	9:00 a. m.	D	5-24	150	−	−	−	−	−	−		None.	
38	3	A. T. & S. F.	9:10 a. m.	D	5-24	1,200	−	+	+	+	−	+	+	6	
39	110	Union Pacific	9:15 a. m.	A	5-24	1,800	+	+	+	+	+	+	+	10	
40	102	Mo. Pacific	9:20 a. m.	A	5-24	700	+	+	+	−	−	+	+	6	
41	518	A. T. & S. F.	9:40 a. m.	D	5-24	100	−	−	−	−	−	−		None.	
42	112	Frisco	9:59 a. m.	A	5-24	1,250	−	−	−	−	−	+	+	1	
43	9	A. T. & S. F.	9:20 a. m.	A	5-24	950	+	+	−	−	+	+	+	6	
44	11	Rock Island	10:10 a. m.	D	5-24	500	−	−	−	−	−	−		None.	
45	110	A. T. & S. F.	—— a. m.	A	5-24	400	−	−	−	−	−	−		None.	
46			—— a. m.		5-24	500	−	−	−	−	−	+	+	1	
47			—— a. m.		5-24	900	−	−	−	−	−	+	+	1	
48	531	Mo. Pacific	4:05 p. m.	D	5-24	75	−	−	−	−	−	−	−	None.	
49	105	Union Pacific	4:10 p. m.	D	5-24	1,200	−	−	−	−	−	−	−	None.	
50	1316	Frisco	4:15 p. m.	A	5-24	90	−	−	−	−	−	−		None.	
51	111	A. T. & S. F.	4:15 p. m.	A	5-24	1,200	−	−	−	−	−	−		None.	
52	109	A. T. & S. F.	4:30 p. m.	A	5-24	800	−	−	−	−	−	−		None.	
53	131	Mo. Pacific	4:30 p.	D	5-24	850	−	−	−	−	−	−	−	None.	
54	5	A. T. & S. F.	9:40 p. m.	D	5-24	30	−	−	−	−	−	−		None.	Chair car.
55	5	A. T. & S. F.	9:40 p. m.	D	5-24	25	−	−	−	−	−	−		None.	Smoking car.
		Tap			5-24	20	−	−	−	−	−	−		None.	Station.
		Union Pacific*			5-24	30	−	−	−	−	−	−		None.	

*Sand springs water.

FROM WICHITA, KAN., MAY 29 AND 30, 1912.

Sample No.	Train No.	Name of Railroad.	Time.	Train arriving or departing.	Date, 1912.	Bacteria per cc. on agar at 37° C, 24 hours.	Gas in lactose bile.							Approximate number of *B. coli* per 10 cc.	Remarks.
							1 cc.					10 cc.	Confirmatory test.		
							1	2	3	4	5				
1	12	A. T. & S. F.	12:26 a. m.		5–30	130	−	−	−	−	−	−		None.	
2	32	Rock Island	12:55 a. m.		5–30	150	−	−	−	−	−	−		None.	
3	403	Mo. Pacific	7:30 a. m.		5–30	400	−	−	−	−	−	−		None.	*B. pyocyaneus* present; iced at Yates Center.
4	433	Mo. Pacific	7:50 a. m.		5–30	300	−	−	−	−	−	−		None.	*B. pyocyaneus* present; iced and H_2O at Wichita.
5	703	Mo. Pacific	7:45 a. m.		5–30	450	−	−	−	−	−	−		None.	H_2O at Wichita.
6	117	A. T. & S. F.	8:20 a. m.		5–30	1,200	+	+	+	+	+	+	+	10	
7	309	Frisco	8:25 a. m.		5–30	300	−	−	−	−	−	−		None.	Car 309. Iced at Minette and Walire.
8	309	Frisco	8:25 a. m.		5–30	150	−	−	−	−	−	−		None.	Car 1016.
9	517	A. T. & S. F., Englewood branch	8:30 a. m.	D	5–29	550	+	+	+	+	+	+	+	10	Car 137; iced and H_2O at Wichita.
10	1	Orient	8:35 a. m.	D	5–29	500	+	+	+	−	−	+	+	6	Iced and H_2O at Wichita.
11	12	Rock Island	9:50 a. m.		5–29	650	−	+	+	+	+	+	+	8	Iced at Caldwell; H_2O at Chickasha, Okla.
12	406	A. T. & S. F.	10:45 a. m.		5–29	500	−	−	−	−	−	−	−	10	Iced at Pratt, also water.
13	406	A. T. & S. F.	11:10 a. m.		5–29	600	+	+	−	−	−	+	+	4	Iced at Arkansas City; H_2O at Purcell.
14	8	Frisco	11:15 p. m.		5–29	600	−	−	−	−	−	−		None.	Car 62.
15	8	Frisco	11:15 p. m.		5–29	550	−	−	−	−	−	−		None.	Car 1030.
16	16	A. T. & S. F.	1:50 p. m.		5–29	100	−	−	−	−	−	−		None.	
17	518	A. T. & S. F., Englewood branch	1:30 p. m.	A	5–29	1,200	+	+	+	+	−	+	+	8	
18	505	A. T. & S. F.	5:25 p. m.		5–30	350	−	−	−	−	−	−		None.	*B. pyocyaneus* present.
19	710	Mo. Pacific	6:20 p. m.		5–30	300	−	−	−	−	−	−		None.	H_2O at Nevada; Ice Coffeyville.
20	405	Mo. Pacific	6:25 p. m.	A	5–30	350	−	−	−	−	−	−		None.	
21	2	Orient	7:00 p. m.	A	5–30	450	−	−	−	−	−	−		None.	
22	24	Rock Island	9:20 p. m.		5–30	500	+	+	+	+	+	+	+	10	*B. pyocyaneus* present.
23		Tap (city)	8:00 a. m.			60	−	−	−	−	−	−		None.	Tap at depot A. T. & S. F.
24	2	Midland Valley	8:00 a. m.	D		400	+	−	+	+	−	+	+	6	Iced and H_2O at Wichita.
25		Tap				90	−	−	−	−	−	−		None.	Tap at Mo. Pacific.
26		Tap				80	−	−	−	−	−	−		None.	Tap at Rock Island.
27		Tap				60	−	−	−	−	−	−		None.	Tap at Frisco.

Altogether there were seventy-eight samples taken, and in order to simplify matters these have been rearranged into four groups according to the degree of pollution, beginning with the worst samples in group I and ending with the purest water in group IV.

Group I includes all those samples having at least 10 colon organisms in 10 cc. of water, or 1 per cc. of the water examined, and is as follows:

GROUP I.

City.	Sample No.	Train No.	Name of Railroad.	Time.	Arriving or departing.	Date.	Count per cc. on agar at 37° C., 24 hours.	Approximate number of *B. coli* in 10 cc.
Kansas City, Mo.	39	110	Union Pacific	9:15 p. m.	A	5–24	1,800	10
Wichita	6	117	A. T. & S. F.	8:20 p. m.	D	5–29	1,200	10
Wichita	9	517	A. T. & S. F., Englew'd branch,	8:30 a. m.	D	5–29	550	10
Wichita	12	506	A. T. & S. F.	10:45 a. m.		5–29	500	10
Wichita	22	24	Rock Island	9:20 p. m.		5–30	500	10

Group II includes those having from 6 to 8 *B. coli* in 10 cc. of water:

GROUP II.

City.	Sample No.	Train No.	Name of Railroad	Time.	Arriving or departing.	Date.	Count per cc. on agar at 37° C., 24 hours.	Approximate number of *B. coli* in 10 cc.
Kansas City, Mo.	1	1	Rock Island	11:15 p. m.	D	5–23	800	6
Kansas City, Mo.	35	111	Missouri Pacific	8:15 a. m.	D	5–24	1,000	8
Kansas City, Mo.	38	3	A. T. & S. F.	9:10 a. m.	D	5–24	1,200	6
Kansas City, Mo.	40	102	Missouri Pacific	9:20 a. m.	A	5–24	700	6
Wichita	1	1	Orient	8:35 a. m.	D	5–29	500	6
Wichita	11	12	Rock Island	9:50 a. m.		5–29	650	8
Wichita	17	518	A. T. & S. F., Englew'd branch,	1:30 p. m.	A	5–29	1,200	8
Wichita	24	2	Midland Valley	8:00 a. m.	D	5–30	400	6

Group III: There are fourteen samples having from 1 to 4 *B. coli* per 10 cc. of the water. In two of them both *Streptococcus pyogenes* as well as *B. coli* was present.

Group IV: There are forty-nine samples in which no *B. coli* were present in 10 cc. of the water.

In group I there are five samples, or 6.4 per cent, containing 10 colon bacteria in 10 cc. of the water, or 1 per cc., with bacterial counts ranging from 500 to 1800 per cc. In one sample *Bacillus pyocyaneus*, the green-pus organism, was found to be present. Waters in this group are decidedly unfit for drinking purposes.

In group II there are nine samples, or 11.4 per cent, in which there are 6 to 8 colon organisms in each ten cc. of the water, with

the bacterial count ranging between 400 and 1200 per cc. These waters are open to strong suspicion and should not be drunk.

In group III there are fourteen samples, or 17.8 per cent, having from 1 to 4 *B. coli* in each 10 cc. of the water, with comparatively high counts.

Of the forty-nine samples in which no *B. coli* was found, twenty-two of them, or 44.9 per cent, show counts developing at body temperature of over 350 bacteria per cc. and eighteen of these have 500 or more bacteria per cc. Thirty-one samples show counts under 500 per cc., and of these twenty-seven, or about one-third of the total number of samples examined, are under 350 per cc. That is, if we attribute any significance—and we must—to the number of bacteria developing at body temperature, and adopt even 350 per cc. as a dividing line between clean and dirty water, we find that of the seventy-eight samples examined, only twenty-seven, or about one-third, showed themselves to be sufficiently clean for drinking purposes.

The possible sources of pollution in these samples of water may be grouped as follows:

1. From the original water supply.
2. From the water used in the ice.
3. From the dirt adhering to the ice.
4. From the hands or person of the individual handling the ice.
5. From the buckets, wheelbarrows, etc., used in carrying and handling the ice.
6. From carelessness in washing the tanks as to thoroughness and as to kind of water used in washing.

The following data as to the water and ice furnished passenger trains in Kansas was supplied by the railroads to Doctor Crumbine:

Railroad and Station.	Water or ice.	Source.	Method of handling.
Rock Island:			
Armourdale	Water.	Railroad plant	Buckets and tongs.
Armourdale	Ice	Manufacturing plant	Buckets and tongs.
Horton	Water.	Well	
Horton	Ice		Tongs.
Topeka	Water.	City	
Topeka	Ice	Manufacturing plant	Buckets.
McFarland	Water.	Well	
McFarland	Ice	Manufacturing plant	Buckets.
Herington	Water.	Railroad plant	
Herington	Ice	Manufacturing plant	Tongs.
Caldwell	Water.	City	
Caldwell	Ice	Manufacturing plant	Buckets.
Liberal	Water.	Railroad plant	
Liberal	Ice	Manufacturing plant	Buckets.
Pratt	Water.	Railroad plant	
Pratt	Ice	Manufacturing plant	Buckets.
Belleville	Water.	City	
Belleville	Ice	Natural	Buckets.
Goodland	Water.	Railroad plant	
Goodland	Ice	Manufacturing plant	Buckets.
Phillipsburg	Water.	Piped from Glade	
M. K. & T.:			
Parsons	Water.	City	
Parsons	Ice	Manufacturing plant	
Kansas City	Water.	City	
Junction City	Water.	City	
Junction City	Ice	Manufacturing plant	
Iola	Water.	City	
Iola	Ice	Manufacturing plant	
Union Pacific:			
Armstrong	Water.	City	
Armstrong	Ice	Natural and manufactured.	
Manhattan	Water.	Railroad plant	
Manhattan	Ice	Natural and manufactured.	
Junction City	Water.	City	
Junction City	Ice	Natural and artificial	
Solomon	Water.	Railroad plant	
Solomon	Ice	Natural and manufactured.	
Salina	Water.	Railroad plant	
Salina	Ice	Natural and manufactured.	
Ellis	Water.	Railroad plant	
Ellis	Ice	Natural and manufactured.	
Leavenworth	Water.	City	
Leavenworth	Ice	Natural and manufactured.	
Onaga	Water.	Railroad plant	
Onaga	Ice	Natural and manufactured	
Miltonvale	Water.	Railroad plant	
Miltonvale	Ice	Natural and manufactured.	
Belleville	Water.	Railroad plant	
Belleville	Ice	Manufactured	
Beloit	Water.	Railroad plant	
Beloit	Ice	Manufactured	
Orient:			
Wichita	Water.	Railroad plant	
Wichita	Ice	Manufactured	
Concordia	Water.	Railroad plant	
Concordia	Ice	Natural	Buckets.
Atchison	Water.	City	
Atchison	Ice	Natural	Buckets.
Oberlin	Water.	Railroad plant	Put in tanks by roundhouse men.
Oberlin	Ice		
St. Francis	Water.	Railroad plant	Put in tanks by roundhouse men.
St. Francis	Ice		

All of the water supplies investigated showed themselves to be good, potable water. An inspection of the ice used in the coolers quite often revealed the presence of dirt in artificial as well as natural ice.

The presence of *B. pyocyaneus* is of no great significance, as organisms closely resembling or identical with it are often found in normal waters. Its source could not be determined, but was probably from the air or dirt. No cuts or wounds were found on the hands of those handling the ice, water or coolers. As mentioned above, one must not lay too much stress on a body-temperature count alone, yet from the data obtained at Kansas City it will be noted that of those trains watered at Kansas City, nearly 20 per cent had counts under 100 per cc., and 12 per cent had counts of 40 per cc. or less, while the sources of supply showed a count of 20 per cc. with no *B. coli* present in 10 cc. From this data, and after making a sanitary survey of conditions, it seems that there is no excuse for so many samples having body temperature counts of over 350 per cc., and that these high counts show carelessness in cleaning the coolers and handling the ice.

The publication of this paper at this time has been made because of its historical interest and because of numerous requests for reprints of the report. It should be understood that these conditions probably in no way exist to-day since the federal government and the state of Kansas have carefully carried out investigations, and laws have been passed regulating the handling of drinking water on common carriers. This investigation which Doctor Crumbine had carried out was, perhaps, the first of its kind in the United States. As a result of it, the Kansas State Board of Health formulated rules and regulations governing drinking water on trains, which antedated the federal government's action over two years. He made use of this and other data at his command in his pioneer work for the betterment of conditions of drinking water on common carriers.

Since this investigation was made much research work and improvement has been done along lines of water investigation. The value of lactose bile has been questioned because of its inhibitive action on the colon group, and some interesting work along the line of differentiation between fecal and other strains of bacillus coli has been carried out.

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CONTENTS:

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A Study of the Relative Efficiency of the Various Differential Staining Methods Used in Identifying the Tubercle Bacillus.*

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THERE has been much discussion as to the classification of the tubercle, leprosy, smegma, grass, and butter bacilli. Migula classifies them with the Bactereaceæ, while Abbot and others have placed them among the trichobacteria. They differ from the rest of the bacteria in that they contain fats, waxes and alcohols, which render them quite difficult to stain with the ordinary basic anilin dyes, such as methylene blue, basic fuchsin, or gentian violet. By covering a dried and fixed preparation of these bacteria on a slide with basic fuchsin or gentian violet, and steaming it from three to five minutes over a free flame, the stain is taken up and retained by these organisms with much greater tenacity than practically any other members of the bacterial kingdom. In fact, when fuchsin is steamed into them they resist decolorization by three to five per cent aqueous solution of any of the mineral acids for thirty minutes to an hour or even longer, whereas other bacteria are decolorized by this treatment. For this reason the tubercle, leprosy, smegma, grass, and butter bacilli are called the acid-fast group of bacteria. Jordan[1] says that this property is due largely to the presence of mykol, one of the higher alcohols. Spores of such organisms as *Bacillus subtilus* and *Bacillus anthracis* are also acid-fast, although the respective organisms are not.

This property of resistance to decolorization with mineral acids, when once the organisms have the stain steamed into them, has

* Read before Kansas Academy of Science January, 1916.

been taken advantage of by bacteriologists and made use of in the routine laboratory examination of sputum, urine, pus, etc., for tubercle bacilli in suspected cases of tuberculosis, and by many in examining butter, milk and other foods for the presence of tubercle bacilli. Now, as was stated above, this acid-fastness or acid-proofness is not the property of just one organism but of a small group of organisms. It has been the aim of many workers to find some method of decolorization that would enable one to differentiate between true tubercle bacilli and other members of this group. This seems to be desirable in order to eliminate possibilities of error in the laboratory examination of suspected tuberculous material, since the smegma bacillus is very commonly found in normal smegma, and has been reported as found, occasionally, in gangrenous conditions of the lungs, on the tonsils, in the tartar from the teeth, from scrapings from the tongue, and on the skin in the inguinal and axillary regions. The butter bacillus and grass bacillus may be present in butter, in other dairy products, and foods. Hence, either of these last two organisms may be found present in the mouths of normal individuals. Since tuberculosis is caused only by the tubercle bacillus and not by any other members of the acid-fast group, it is desirable when examining suspected material to rule out possibilities of error due to the presence of these other organisms. Many staining methods have been suggested, tried and recommended. They may be grouped into five groups as to theories and methods:

1. That true tubercle bacilli differ from the rest of the acid-fast group in their resistance to decolorization with 25 or 30 per cent aqueous solution of the mineral acids. All of the acid-fast organisms, excepting true tubercle bacilli, are said to be decolorized.

2. That while this group is acid-fast, only true tubercle bacilli are acid-alcohol fast. Hence, acidulated alcohol (2 to 3 per cent hydrochloric or nitric acids in from 70 per cent to absolute alcohol) has been used as a differential agent.

3. Some modification of (2), such as Pappenheim's, where rosalic acid is used in connection with glycerine and absolute alcohol.

4. That it is necessary and important to remove the fats and use strong concentrations of mineral acids and absolute alcohol to decolorize everything except the tubercle bacillus.

5. Fonte's method, which is a combination of the acid-fast and Gram's stain, as described below.

As a method typifying the first theory, Gabbet's[2] is perhaps the best. In this method the preparation is made and stained with steaming carbol-fuchsin from three to five minutes. The excess of staining fluid is drained off without washing and is replaced by Gabbet's methylene blue solution (2 gr. M. B.; sulphuric acid, 25 cc.; water, 75 cc.). This solution is allowed to act for from one to three minutes and is then washed off with water and the preparation dried and examined. The tubercle bacilli will appear as bright red rods, while all the other organisms are said to be stained blue.

The Ziehl-Neelsen[3] method comes under the second group. In this method the organisms are stained with steaming carbol-fuchsin from three to five minutes and decolorized with acidulated alcohol for several minutes, or until no more color comes from the slide. It is then washed in water and counter-stained with Loeffler's methylene blue. The tubercle bacillus will appear red, while all the other bacteria are supposed to be blue.

A modification of this method, which is reported to have given greater efficiency, is Pappenheim's[4] method. The preliminary staining is carried out as above. The specimen is then drained and covered with decolorizing solution, which is made by dissolving one gram of rosalic acid in 100 cc. of absolute alcohol, saturating the mixture with methylene blue and adding twenty parts of glycerine. The slide is then washed in water, dried between blotting paper, and examined with the immersion lens. The tubercle bacilli are stained red and all the other organisms are said to be blue.

As an example of group IV is Bunge and Trantenroth's[5] method. After fixation of the smear the fat is removed by soaking the specimens in absolute alcohol. The preparation is now covered with a 5 per cent solution of chromic acid for fifteen minutes, after which it is washed with water. The smear is stained with steaming carbol-fuchsin, decolorized with 16 per cent sulphuric acid for three minutes, and is then counter-stained for five minutes in a concentrated alcoholic solution of methylene blue. This method is said to give the tubercle bacillus a distinct red color, while the smegma bacillus is blue.

Fonte's[6] method for the differentiation of the tubercle bacillus from the rest of the acid-fast group is as follows:

1. Stain the dried and fixed preparation with steaming carbol-fuchsin for three minutes.
2. Wash with tap water.

3. Stain in the cold, with carbol-gentian violet, for two or three minutes.

4. Drain off the stain and treat with Lugol's solution until no more metallic mirrors appear. Blot dry with filter paper.

5. Decolorize with absolute alcohol and ether (equal parts) until no more color comes from the slide.

6. Counter-stain with methylene blue.

7. Wash in distilled water and dry, and examine.

The tubercle bacilli appear as bright red rods containing blue granules. The other members of the acid-fast group, as well as other Gram positive organisms, appear Gram positive.

The scope of this paper is an investigation of the value of Gabbet's, Ziehl-Neelsen's, Pappenheim's, Bunge and Trantenroth's, and Fonte's methods as a means of differentiating between *Bacillus tuberculosis*, *B. lepræ*, *B. smegma*, Moeller's grass bacillus, and the butter bacillus.

This work was undertaken because of the various conflicting statements in the literature as to the value of these solutions in differentiating between true tubercle bacilli and the rest of the acid-fast group; because Pappenheim's, Bunge and Trantenroth's, and Ziehl-Neelsen's methods are accepted and used in many laboratories as giving a reliable differentiation.

Alvarez and Travil[7] (1885), Klemperer and Bittu,[8] and Cowie[20] (1900-'01), as well as others, have described peculiar bacilli in smegma taken from the genitals of man and the lower animals, as well as from moist skin in the folds of the groin, the axilla and the arms. They also mention its presence in normal urine and sometimes in saliva and sputum. They are described as morphologically resembling the tubercle bacillus and resisting decolorization by mineral acids, but being decolorized by alcohol.

Moeller[9] (1898) found in milk, butter, timothy hay, cow dung, etc., acid-fast organisms. They were acid-alcohol-fast but non-pathogenic for laboratory animals. Moeller (1902) cultivated acid-fast organisms from the skin. He concluded that all acid-fast organisms that he had worked with were tinctorially like the true tubercle bacillus.

Marzinowski[10] (1900) found saprophytic acid-fast organisms associated with bronchitis. Frinkel[11] (1898), and Rabinovitch[13] (1900), found acid-fast organisms associated with pulmonary gangrene; they were saprophytes. Petri (1897), Rabinovitch[12] (1899) and Korn[14] (1899) have described as *Bacillus butyricus* an acid-fast organism, morphologically like the tubercle bacillus,

which may at times be found in butter. They think it would be mistaken for the tubercle bacillus from its morphology and staining reactions. It could be differentiated on cultural grounds, inasmuch as it grows readily on artificial media, whereas the tubercle bacillus does not.

Abbot and Gildersleeve[15] (1902) concluded from their study of the acid-fast group that 30 per cent nitric acid in water is a fairly satisfactory method of differentiation. They state that the majority of acid-fast bacteria are unable to resist decolorization with this nitric acid solution, whereas it does not affect the stain in the tubercle bacillus. They would also place the acid-fast organisms in the group of *Actinomycetes*. In regard to animal inoculation, they state that some of the acid-fast bacteria other than the tubercle bacillus are capable of causing in rabbits and guinea pigs nodular lesions suggestive of tubercles; that these lesions, while often very much like tubercles in their histological structure, may nevertheless be distinguished from them by the following peculiarities:

A. When occurring as the result of intravenous inoculation, they are always seen in the kidneys, only occasionally in the lungs, and practically not at all in the other organs.

B. They constitute a localized lesion, having no tendency to dissemination, metastasis, or progressive destruction of tissue, by caseation.

C. They tend to terminate in suppuration or organization rather than in progressive caseation as in the case with tubercles.

D. They are more commonly and conspicuously marked by the *Actinomycetes* type of development of the organisms than is the case with true tubercles, and these *Actinomycetes* are less resistant to decolorization by strong acid solutions than are those seen in tubercles.

For the experimental work of this paper, cultures of the various members of the acid-fast group were obtained from year to year, from the American Museum of Natural History. A culture of the tubercle bacillus was obtained from the University of Michigan and one from the University of Chicago. Two cultures (two strains) of the leprosy bacillus were obtained from Tulane University. Sputums from clinical cases of tuberculosis were also examined. In addition to the pure cultures of *B. smegmatis* obtained from the American Museum, normal smegma was secured from a number of individuals and used in these experiments.

Several samples of butter were also examined for the butter bacillus.

The pure cultures were carried along on glycerine agar, plain agar, and in glycerine broth. Cultures varying from one to three weeks in age were found satisfactory.

In studying the relative efficiency of Gabbet's, Pappenheim's, acid-alcohol, etc., as decolorizing agents the standard was the number of acid-fast organisms present when decolorized with 3 per cent nitric acid in water. Several slides were run on each of these as controls and in making estimates.

In the following table four plus (+ + + +) indicates complete acid-fastness with the reagent used, while a minus sign (—) indicates non-acid-fastness. The intermediate degrees are indicated by the number of plus signs, varying between the two limits. Where only a few organisms retain the stain, and that only to a slight degree, the double dagger is used (††).

DATA.

Source of Organism.	Staining and decolorizing methods—counter-stained with methylene blue.					
	3 per ct. nitric acid in water.	Gabbot's.	Pappenheim's.	Acid alcohol.	Bunge and Tranteroth's.	Fonte's.
B. tuberculosis.						
1. Cult., A. M., '12	++++	++++	++++	++++	++++	
2. Cult., A. M., '13	++++	++++	++++	++++	++++	
3. Cult., A. M., '14	++++	++++	++++	++++	++++	–
4. Cult., A. M., '15	++++	++++	++++	++++	++++	–
5. Cult., U. of M	++++	++++	++++	++++	++++	–
6. Cult., U. of C	++++	++++	++++	++++	++++	–
7. Sputums from 29 positive cases	++++	++++	++++	++++	++++	+++††
8. Sputum	++++	++++	++++	++++	++++	††
9. Urine	++++	++++	++++	++++	++++	††
10. Pus from cervical lymph glands	++++	++++	++++	++++	++++	++++
B. leprx.						
1. Cult., A. M., '13	++++	++++	+++††	++++	+++††	
2. Cult., A. M., '14	++++	++++	+++††	++++	+++††	–
3. Cult., A. M., '15	++++	++++	+++††	++++	+++††	–
4. (2) Cult., Tulane	++++	++++	+++††	++++	+++††	–
B. smegmatis.						
1. Cult., A. M., '12	++++	++++	+++	++++	++	
2. Cult., A. M., '13	++++	++++	+++	++++	++	
3. Cult., A. M., '14	++++	++++	++††††	++++	++††	–
4. Cult., A. M., '15	++++	++++	+++††	++++	+++††	–
5. Cult. from normal smegma (a)	++++	+++	††	++	+††–	–
6. Normal smegma smears	Many acid-fast.	++++	††	++	+††	–
7. Cult. from normal smegma (b)	++++	+++	–––	††	††	–
8. Normal smegma smears (b)	Many acid-fast.	+++	–	††	+	–
9. Normal smegma smears (c)	A few acid-fast.	–	–	–	–	–
10. Normal smegma smears	A few acid-fast.	–	–	–	–	–
11. Normal smegma smears	Many acid-fast.	++++	††	+††	+††	–
12. Normal smegma smears	A few acid-fast.	††	–	–	–	–
13. (15) Normal smegma smears	None acid-fast.	–	–	–	–	–
B. Rabinovitch. (*Butter bacillus.*)						
1. Cult., A. M., '12	Many acid-fast.	+++	+++††	++++	+++	
2. Cult., A. M., '13	Many acid-fast.	+++	+++††	++++	+++	–
3. Cult., A. M., '14	Many acid-fast.	+++	+++††	++++	+++	–
Moeller's grass bacillus.						
1. Cult., A. M., '12	Many acid-fast.	+++	+++†††	++++	+††	–
2. Cult., A. M., '13	Many acid-fast.	+++	+++	++++	++	–
3. Cult., A. M., '14	Many acid-fast.	+++	+++††	++++	+††– –	–
4. Cult., A. M., '15	Many acid-fast.	+++	+++††	++++	+††	–

DISCUSSION.

It will be observed that by Fonte's method all of the members of the acid-fast group of organisms growing on culture media saprophytically were decolorized. This includes the pure cultures of the tubercle bacillus. None of the other methods would completely decolorize any of the members of this group of organisms. Bunge and Trantenroth's and Pappenheim's having as a rule a greater efficiency than Gabbet's or the Ziehl-Neelsen method, but even with these there were always some organisms of each member of the group retaining the fuchsin stain. On the other hand, in twenty-nine positive sputums from clinical cases of tuberculosis the tubercle bacilli remained acid-fast by Fonte's method as well as by the other methods. One sample of sputum from a doubtful case of tuberculosis (8), however, which when examined by Fonte's method showed only a few weakly acid-fast organisms containing the characteristic blue granules, appeared entirely different by the Ziehl-Neelsen and other methods, showing by these fifty and seventy-five strongly acid-fast organisms per field. This sputum was treated for a few minutes with 15 per cent antiformin, and after repeated washings the sediment was injected into guinea pigs. The injected sediment was rich in acid-fast organisms by the Ziehl-Neelsen method. After three months the pigs were normal in weight, did not respond to the tuberculin test, and at *post mortem* showed no evidence of tuberculosis except for the slight enlargement of a few mesenteric lymph glands. The tissue examination was negative. A similar report can be made in regard to urine sample (9), where many strongly acid-fast organisms were found by Gabbet's, Ziehl-Neelsen's, Pappenheim's, and Bunge and Trantenroth's methods, while only a few very weakly acid-fast organisms were in evidence by Fonte's method. Animal inoculation gave negative results. Clinically this case is not tuberculosis at present. In regard to the action of these two strains of acid-fast organisms one of the following must be true: (1) That the organisms are not tubercle bacilli; (2) that if they are they are so avirulent as not to cause tuberculosis in guinea pigs; (3) that the guinea pigs were unusually resistant to tubercle bacilli; (4) that the bacteria were attenuated or killed by the antiformin. In view of the fact that saprophytic acid-fast organisms have been isolated from sputums and urines, that guinea pigs are considered very susceptible to the tubercle bacillus, that the antiformin did not injure their acid-fast properties and is generally

considered not injurious to acid-fast tubercle bacilli,[16] and since the patients are not positively tuberculous clinically, it would appear that the organisms were probably saprophytes. The action of the organisms towards Fonte's method was a border-line one. In view of the work of Spengler[17] and also of Much[18] on non-acid-fast tubercle bacilli, one can not use this border-line reaction as evidence one way or the other.

It is hoped to continue this work and determine the relative frequency of occurrence of saprophytic acid-fast organisms in urine and sputums and to further check up the value of Fonte's and other promising methods of differentiation. If non-acid-fast or partially acid-fast tubercle bacilli were very commonly met with in urines and sputums from tuberculous patients it would be quite evident that no staining method based upon acid-fastness would be efficient, but there has not been much evidence brought forward to show anything but the scarcity of these non-acid-fast strains. The work of Wherry[19] indicates that this cause of acid-proofness of parasitic organisms is different from that of saprophytes. As to the acid-fast properties of intermediate organisms with a very low grade of virulence nothing has been reported along the line of Wherry's investigation. The many conflicting results which have led to the development of so many differential staining methods, added to conflicting opinions of the acid-proofness of different members of this group, suggests a wide variation in acid-proofness among the saprophytic members of the acid-fast group, at least. The work of this paper further confirms this fact. An explanation for the decolorization by Fonte's method of pure cultures of the tubercle bacillus, as well as other acid-fast organisms as reported in this paper, is that the tubercle bacillus, as well as the others, was growing saprophytically, hence its acid-proofness differed materially from that of the parasitic tubercle bacilli of the twenty-nine positive clinical cases of tuberculosis. These laboratory strains of the tubercle bacillus have also lost their pathogenicity. It may be that the groups are so closely related as to offer insurmountable obstacles to their absolute and complete differentiation. A method having a very high percentage of efficiency would be very valuable and desirable.

CONCLUSIONS.

1. That there is great variation in the acid-proofness of different strains of *B. smegmatis*.

2. That even in positive sputums there is some tinctorial difference of the tubercle bacillus toward Fonte's stain, whereas with the other methods very little if any tinctorial variations were observed.

3. That Gabbet's, Ziehl-Neelsen's, Pappenheim's, and Bunge and Trantenroth's methods are not at all reliable as a means of differentiating the tubercle bacillus from the rest of the acid-fast group.

4. That Fonte's method seems to be much superior to the other methods, but not entirely reliable in urine, and even in sputum examinations. The percentage of error can only be determined by much more extensive work.

5. That the error of all of these methods seems to be that of giving too many positive results.

6. That Fonte's method might prove quite serviceable in urine and feces examinations when used along with the clinical symptoms, cellular content and chemical tests. In case any of the latter are negative, animal inoculation should be resorted to. It must be remembered that none of the above staining methods can be relied upon alone. Probably few errors are made in sputum examination, on the positive side, by any of the routine methods, because clinical symptoms are usually pronounced before the sputum is examined. Perhaps many more sputums are called negative that should be positive than the reverse. However, there is at least some chance for error on the positive side in sputum examinations. With urine and feces the great problem confronting the bacteriologist is to rule out the saprophytes.

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CONTENTS:

ON A COLLECTION OF FOSSIL VERTEBRATES MADE BY DR. F. W. CRAGIN FROM THE EQUUS BEDS OF KANSAS. . . *Oliver P. Hay.*

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On a Collection of Fossil Vertebrates Made by Dr. F. W. Cragin in the Equus Beds of Kansas.*

BY OLIVER P. HAY.

IN the sixth volume of the Colorado College Studies, on pages 53 and 54, issued at Colorado Springs, Colo., March, 1896, Dr. F. W. Cragin made a brief report on some vertebrate fossils which he had discovered in 1891, in Meade and Clark counties, Kansas. Later Doctor Cragin became interested in other studies and did not come back to these fossils. These now form a part of the collection of Colorado College, and, through the kindness of the honorary director of the museum, Mr. Edward R. Warren, the writer has been permitted to bring them to Washington and to study them.

A portion of the fossils were found near the head of Bluff creek, in the western part of Clark county, east of Minneola, and the others along Spring creek, a tributary of Crooked creek, about four miles southwest of Meade, in Meade county. In this region Doctor Cragin discovered three terranes, the lowest of which, varying from ten to forty feet in thickness, he named the Meade gravels. He recognized that these gravels belonged to what has been called the Equus beds. From them he reported *Elephas imperator* (?), *Megalonyx leidyi*, *Equus complicatus*, *E. curvidens*, and *Auchenia huerfanensis*.

Overlying the Meade gravels there was found a bed of volcanic ash which reached a thickness of thirteen feet. This contained few fossils. It was named the Pearlette. Above this ash bed were found marls, to which was applied the name Kingsdown. In that region the marls were apparently not less than 100 feet thick. Only *Elephas* was reported from them.

All of the materials of the collection made by Cragin which have been studied by the writer belong to the lowest terrane, the

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Meade gravels. The following species have been determined, of which two are new:

Testudo equicomes, new species.
Mylodon harlani Owen.
Hipparion cragini, new species.
Equus complicatus Leidy.
E. leidyi Hay.
Camclops huerfanensis (Cragin).
Canis occidentalis? Richardson.
A large undetermined felid.

Testudo equicomes, new species.

In the Cragin collection made on Spring creek in Meade county there are eight fragments of bones which belonged to species of the genus *Testudo*. It is quite certain that three or four species are indicated, but only two of the fragments can be assigned to the same individual or species. To these the name *Testudo equicomes* is given. The other pieces of bone can not be assigned to their species.

The two bones which quite certainly belonged to the same individual are the right epiplastron and the left hypoplastron. The epiplastron (pl. I, figs. 1, 2) has a projecting and much thickened beak. The distance from the midline in front to the point on the free border where the bone joined the hypoplastron is 68 mm. The width of the bone at the middle of its length is 30 mm. The length of the beak, measured on its upper surface and at the midline, is 35 mm.; and at its rear it is 27 mm. thick. The whole free border of the bone is acute. From this border the hinder half of the bone thickens until, at a short distance behind the beak, it reaches a thickness of 11 mm.

The hinder and inner borders of the hypoplastron (pl. I, fig. 3) are missing, so that the bone present reaches neither the midline nor the hypoplastron. From the anterior end of its free border to the axillary notch is a distance of 30 mm.

Figure 1 of plate III represents an attempt to restore the anterior portion of the plastron from the two fragments described above. The lobe appears to have been relatively short and broad. From one axillary notch to the other was apparently 160 mm. From the middle of the line joining these notches to the front of the beak was 66 mm. The entoplastron appears to have been unusually wide, apparently about 80 mm. How it ended behind is conjectural. From the outer edge of one hypoplastron, at the bridge, to that of the other was about 200 mm. Basing the esti-

mates on this width it is concluded that the carapace had a length of about 340 mm. and a width of 290 mm.

As will be observed on the figures, the gular scutes extended backward on the entoplastron. The humeropectoral sulcus bends backward and again forward as it crosses the hypoplastron. This forward curve makes it probable that the sulcus crossed the hinder end of the entoplastron, but this is not certain. Another sulcus just outside of the axillary notch cut off an axillary scute.

This tortoise is quite distinct from *Gopherus polyphemus*. It is probable that a very large species, *T. crassiscutata*, inhabited Kansas and the region further south during the early Pleistocene, inasmuch as it occurs in Texas; but this was far larger, and the bones described above give no indication of having been those of a young animal.

Mylodon harlani Owen.

In the collection are three teeth of a *Mylodon* which seem to belong to the species named above. Cragin reported these as belonging to *Megalonyx*; but they certainly do not belong to that genus. They were found four miles southwest of Meade, Kan.

These teeth are identified as the left first and third upper teeth and the right first of the lower jaw. They are all damaged somewhat and parts have been lost at some time since exhumation.

The first upper tooth is shown on plate I, figure 4; and a section is shown on the plate (pl. III, fig. 2). The present length of the tooth, measured along the greater curve, is 120 mm. Originally it was somewhat, but not greatly, longer. It will be observed that it curved considerably in passing downward and forward from the bottom of the socket. In section the tooth is broadly oval, with the inner face somewhat flattened. The anteroposterior diameter is 21 mm.; the transverse, 17 mm. It will be seen that this resembles closely the section of the first tooth given by Cope (Proc. Amer. Philos. Soc., vol. XXXIV, p. 459, pl. X, fig.1,*a*) and reproduced by the present writer (Geol. Surv. Iowa, vol. XXIII, p. 134, fig. 33). The grinding surface is concave from front to rear and it slopes somewhat downward from the outer side.

The third upper tooth is here shown in section (pl. III, fig. 3). The present height of the tooth is 70 mm., but it was originally higher. The greatest diameter is 36 mm., the width across the anterior lobe, 27 mm. The hinder lobe is 13 mm. thick. The grinding surface shows two planes of wear, one on the broad an-

terior lobe, the other on the hinder lobe. The two meet at the place of union of the two lobes, making an angle of about 135°. This tooth appears to resemble closely the third upper tooth of G. M. Allen's *Mylodon garmani* (Mem. Mus. Comp. Zool., vol. XL, pl. 2, fig. 3) rather more than that represented by Cope (op. cit.); but the latter tooth was not complete, and was pretty certainly incorrectly restored.

The first lower tooth is here shown from behind (pl. I, fig. 5) to illustrate its curvature, and in section (pl. III, fig. 4). The height in a straight line is 90 mm. The anteroposterior diameter is 23 mm.; the transverse 16 mm. The groove along the inner face is deeper than in the section given by Cope. Of this tooth in *Mylodon garmani*, Allen says that the inner side is nearly flat. As in the tooth just referred to, there are two planes of wear, an anterior and a posterior.

Hipparion cragini, new species.

In the collection made by Doctor Cragin there is an upper molar or premolar which seems to belong to a hitherto undescribed species. Accompanying the specimen is a note made by Doctor Cragin which reads as follows:

"Upper part of Bluff Cr., Clark Co., east of Minneola (Thomas ranch), in Pleistocene deposits bearing *Elephas columbi* and *Equus curvidens*. Can this have been derived from the Loup Fork? Latter occurs, but further east."

It is, of course, possible that the tooth was originally left in deposits preceding the Pleistocene. However, it presents no signs of being water-worn, rolled or weathered. It is stained just as are teeth of *Equus* from the same beds.

It is not yet certain that species of *Hipparion* continued on into the Pleistocene; but there are some indications that they did. A tooth of *Hipparion*, referred by the writer with doubt to *Neohipparion gratum* (Geol. Surv. Iowa, vol. XXIII, pl. IX, figs. 1, 2), was found in what is supposed to be Aftonian gravels, at Rockport, Mo. Other remains were discovered in Aftonian gravels near Thayer, Iowa. The occurrence of the tooth here described in Pleistocene deposits in Kansas supports the view that the genus did not become extinct at the close of the Tertiary.

The crown (pl. I, figs. 6, 7) has now a height of 38 mm. on the outer face. The length of the grinding surface is 17 mm.; the width, 17 mm. The protocone has a fore-and-aft diameter of 6.5 mm. At a distance of 25 mm. above the grinding surface the fore-

and-aft diameter of the crown is 16 mm.; the side-to-side diameter, 17.5.

The outer styles are strongly developed, standing out boldly from the face of the tooth. Near the grinding surface these styles have a diameter of 2.5 mm., but toward the root the median widens to 4 mm. The anterior one also widens slightly.

The lakes have the enamel surrounding them only moderately complicated. In the anterior lake there is a deep infold at the hinder inner angle, and a smaller one in the hinder border. In the hinder lake there are a shallow fold and another rather deep one in the anterior border and a moderate one in the hinder border.

The oval protocone is detached from the other columns of the tooth, but lies close to them. A small loop at the head of the hinder inner valley comes nearly into contact with the enamel of the protocone. The inner face of the protocone is very slightly convex.

It is possible that the *Hipparion* teeth mentioned above as being found in the Pleistocene of Missouri and Iowa belong to this species; but while the crown of the Kansas specimen is very straight, that of the Missouri tooth is considerably curved. The protocone of the latter is considerably smaller than is the former.

This tooth differs from those of *H. gratum*, figured by Cope (Proc. Philos. Soc., vol. XXVI, figs. 16, 17) in being much straighter. From these and most of those figured by Leidy (Jour. Phila. Acad., vol. VII, 1869, pl. XVIII, figs. 25-30) the Kansas tooth differs in having the enamel somewhat more simple in its arrangement.

Equus complicatus Leidy.

In the Cragin collection there are a few teeth which are here referred to this species. Of these there are one upper right premolar and five lower cheek teeth. They were found on Spring creek, near Meade. The upper premolar, the third or the fourth, has a height of 80 mm., a length of 33 mm., and a width of 32 mm. The protocone is 17 mm. wide. There is a deep reëntrant loop at the head of the postprotoconal valley, and the enamel of the lakes is considerably complicated.

Three of the lower teeth appear to have belonged to the same animal. These are the two hinder premolars and the first molar (pl. II, fig. 1). The following are the measurements:

Measurements of lower teeth in millimeters.

Tooth.	Height.	Length.	Width.
Pm_3	58	30	17
Pm_4	65	29	17
M_1	56	25.5	15

The enamel of these lower teeth shows little plication of the enamel, and in that respect they are different from those which are in this paper identified as *Equus leidyi*. These teeth are somewhat smaller than might be expected; however, they are all, and especially the molar, worn down to where the length is somewhat diminished.

Equus leidyi Hay.

In the collection there is a part of the left maxilla, which contains the last premolar and all the molars (pl. II, fig. 2). The specimen was found in the Meade gravels, at the head of Bluff creek, in Clark county. It was regarded by Doctor Cragin as *Equus curvidens* Owen; but the teeth of that South American species have the enamel less complicated and the styles stand out less prominently from the outer face.

The teeth of the Bluff creek specimen are those of a horse probably hardly four years old. The last premolar had been but slightly abraded; the hindermost molar had not yet been cut. The first and second molars are only moderately worn.

The maxilla reaches the midline, thus presenting one-half of the width of the palate, which had, between the first molars, a width of 75 mm. The posterior palatine foramen opened opposite the front half of the second molar. The maxillary ridge continued forward to the middle of the last premolar.

The last premolar is slightly curved; the molars are much curved. As one follows them upward from the grinding surface they bend both backward and inward (pl. II, figs. 3, 4). The following measurements are afforded. The length and the width are taken somewhat above the grinding surface. The dimensions of the last molar are of less importance than those of the other teeth.

Measurements of the cheek-teeth in millimeters.

Tooth.	Height.	Length.	Width.	Protocone.
Pm^3	92	28	29	12
M^1	90	27	27	12
M^2	97	27	26	12
M^3	70	26	24	.....

The styles stand out prominently on the outer faces of the teeth. As usual, the anterior one of the premolar is broader than in the molars, but there is no defined groove running along it. The protocone is slightly concave on its inner face. The enamel of the worn teeth, especially that of the first molar, is considerably complicated. There is a notch in the front wall of the anterior lake, while in the hinder wall of the posterior lake there is a deep infold and two or three shallow ones. The adjacent borders of the two lakes have each a very deep infold and two or three shallower ones. In the head of the postprotoconal valley there is a rather deep reëntrant loop.

Besides the tooth described above there are seven loose upper premolars and molars, which were found along Spring creek, four miles southwest of Meade, Kan. I am not sure that the position of certain of the teeth has been correctly determined. In some cases, as Nos. 2 and 6 of the list below, the anterior style is narrow, indicating that the tooth is a molar, but it is traversed from end to end by a shallow groove. In another case, No. 1, the style is broad and without groove. The following are the measurements. It is to be understood that here, as in all other cases, the height of the tooth is given merely to show the amount of wear it has suffered.

Measurements of teeth in millimeters.

Tooth.	Position.	Height.	Length.	Width.	Protocone.
1	Pm^4	80	26	27	12
2	$Pm^{4?}$	90	24	22	13.5
3	$M^{1?}$	60	23	24	15
4	$M^{1 \text{ or } 2}$	75	24	26	12
5	$M^{1 \text{ or } 2}$	65	26	24	15
6	$M^{1?}$	85	24	24	..
7	Pm^4	62	27	27	12
8	Pm^4	58	28	28	12

In the true molars of *E. leidyi* there appears to be a strong tendency to retain the reëntrant loop at the head of the postprotoconal valley.

Among the lower teeth of horses collected on Spring creek are three premolars (pl. III, fig. 5) which appear to have belonged in the left side of the same jaw; and three molars (pl. III, fig. 6) which apparently belonged in the right side of the jaw of the same individual as that which possessed the premolars, or of another individual of the same age. The horses, if more than one, were young, as will be seen from the height of the various teeth.

Measurements of lower teeth in millimeters.

Tooth.	Height.	Length.	Width.
Pm_2	65	28	13
Pm_3	85	28.5	16.5
Pm_4	88	27	15
M_1	78	25	13
M_2	..	29	15
M_3	60	30	12.5

The premolars are curved so as to be slightly convex on the outer face; the first molar is somewhat convex on the inner face and quite strongly convex on the front border. As will be seen from figure 5, the enamel of the premolars is much crinkled; that of the molars somewhat less so. Attention is called to the fact that the length of the grinding face of premolar 2 is not greater than of the next one.

Both in complication of the enamel and in length and width of the grinding surface these teeth resemble closely the teeth from the region about Charleston, S. C., which have been referred to *E. leidyi*.

In size the upper teeth above described agree closely with those forming the type of *Equus excelsus* Leidy. On close comparison it is seen that the styles on the outer face of the teeth of *E. excelsus* stand out more boldly and the intervening channels are deeper and more concave. The enamel of the lakes is far less complicated .than in the teeth of the specimens collected by Cragin. That this condition is not due to the greater degree of attrition of the former is shown by the fact that some of the teeth of the Cragin collection are still more worn than those of the type of *E. excelsus*, and they still preserve the deep notches and plication of the enamel. In the type of *E. excelsus* the protocone is much longer than in most of the teeth referred to *E. leidyi*. While not too much importance can be attached to this fact, it must be considered. It seems to the writer that the Kansas specimens certainly do not belong to *Equus excelsus*.

Besides the equine materials above described, Doctor Cragin found on Spring creek a median metacarpal. This bone has a length of 225 mm. along the outer border. The width at the upper end is 46 mm.; at the middle of the length, 32 mm.; across the lower articular surface, 39 mm. The bone is very hard and heavy. It has been, at some stage in its history, much weathered, and it is cracked longitudinally. From the same place is another metacarpal nearly identical in dimensions but differently fossilized. Its length along the outer border is 216 mm.; width at the upper end, 46 mm.; at the middle of the length, 31 mm.; across

the lower articular surface, 41.5 mm. A third metacarpal from the same place is larger. The length is 232 mm.; width at the upper end 51.5 mm.; at the middle of the length, 33 mm.; across the lower articular surface 45 mm.

It seems that two species are indicated here; the latter bone being possibly that of *Equus complicatus*, the other two those of *E. leidyi*.

Besides these there are some phalanges, but none that bore hoofs. There are also two astragali. At least two species of horses are indicated in the phalanges and the astragali.

There are in the collection also four moderately worn incisors.

Camelops huerfanensis (Cragin).

Among the fossils collected on Spring creek are several teeth which the writer can not distinguish from those of the type of Cragin's *Camelops huerfanensis*, and it was to this species that Cragin himself referred them. These teeth consist of nine incisors, four canines, three upper second molars, three lower second molars, two lower third molars, two upper milk-molars, and three lower third milk-molars.

The incisors do not differ from those of specimens found at Minidoka, Idaho (Proc. U. S. Nat. Mus., vol. 46, p. 273, pl. 26, fig. 2). A mature canine, probably of the upper jaw, has the crown bent at right angles with the root and furnished with sharp front and rear edges, as in the lama. Another and stouter canine is somewhat less abruptly bent.

The least worn second upper molar has a height of 75 mm., a length of 50 mm. on the outer face at the grinding surface, and a length of 45 mm. at a point two-thirds the distance to the root. At the base the width of the anterior lobe is 28 mm., of the posterior 26 mm.

A right second lower molar, slightly worn, has a height of 70 mm. (pl. II, fig. 5). Its length at the grinding surface is 47 mm.; at two-thirds the way to the root, 40 mm. The width at the base is 19 mm. There is a well-defined style on the front of the inner face and another on its rear. The two lower last molars furnish the following measurements:

Measurements of hindermost lower molars in millimeters.

	No. 1.	No. 2.
Height	70	70
Length near grinding surface	56	62
Length near the root	60	67
Width of front lobe near base	20	23

No. 2 of the preceding table has a quite thick deposit of cement; that of No. 1 has been dissolved off. A second molar (M_2), which belongs with No. 2, has also a deposit of cement.

The two upper milk-molars are each worn only slightly on its front lobe. One (pl. I, fig. 8) has the anterior face broken off, but it was evidently somewhat smaller than the other tooth. Both have prominent external styles, front, median and posterior. The edges of the anterior and median are directed pretty strongly forward. The middle of the outer face of each lobe forms a prominent rounded ridge.

Measurements of upper milk-molars in millimeters.

Tooth.	Height.	Length of summit.	Length near root.	Width at base.
1	43	45±	32	23
2	45	48	32	23

Of the three lower third milk-molars, two belong to the left side, one to the right. One of those of the left side is in a fragment of the jaw (pl. II, fig. 6), and it seems to have belonged to the same individual as the tooth of the right side. Each has a little accessory column, 15 mm. high, just behind the median style of the inner face. This column is absent on the other tooth of the left side. The height of the milk-molar figured is 43 mm.; the length at the summit, 54 mm.; near the base, 40 mm.; width at the base, 16 mm.

On Spring creek there were obtained two upper phalanges of camels. They pertained probably to the front limbs. The following measurements are given to aid in making comparisons with other like materials.

Measurements of phalanges in millimeters.

	No. 1.	No. 2.
Length along outer border	130	115
Width across the upper articulatory surface	49	....
Fore-and-aft diameter at middle of length	29.5	24.5
Side-to-side diameter at middle of length	23	21
Width across lower articular surface	38	32

Canis occidentalis ? Richardson.

In the collection is an injured lower right first molar of a wolf which was found on Spring creek, near Meade. This tooth is referred with some doubt to the species above mentioned. Unfortunately, the outer face of the principal cone and that of the anterior cone have been split off and lost; also the front border of the anterior cone is missing. Comparison of the tooth has been

made with the corresponding one of *Canis dirus*, from Rancho La Brea, California; of *C. occidentalis*, from Alaska; and of *C. gigas*, from Oregon. The following measurements have been taken:

Measurements in millimeters.

	Length of tooth.	Width of talon to length of tooth.	Width of principal cone to length of tooth.
Kansas specimen	32.3	37.1:100	38.7:100
La Brea specimen	36.5	35.6:100	43.8:100
Alaska specimen	32.0	35.3:100	40.6:100
Oregon specimen	28.0	32.1:100	44.6:100

It will be observed that, as regards size, the Kansas tooth is much nearer to that of *C. occidentalis* than to any of the others. The tooth differs from all of the other teeth in the width of the talon. The metaconid is somewhat more strongly developed than in *C. occidentalis*. It is possible that this tooth represents a distinct species, but the evidence is not sufficient to establish this.

Undetermined Felid.

In the collection made on Spring creek, about four miles southwest of Meade Center, Kan., are found some remains of a very large cat-like animal. These consist of the left second metacarpal; the proximal three-fourths of the right fifth metacarpal; the right calcaneum; the proximal two-thirds of the right fourth metatarsal; a proximal phalange of probably the fourth digit of the pes; and the distal half of another proximal pedal phalange. There is no certain evidence that any two of the bones were found together, but it seems probable that all belonged to one individual. They shall here be so considered. All these parts indicate an animal somewhat larger than the African lion.

The second metacarpal, as the other bones, is compared with that of a lioness captured in Africa by the Roosevelt party.

Measurements of second metacarpals in millimeters.

Dimensions Taken.	Fossil felid.	*Felis leo.*	Ratio of fossil to *Felis leo* taken as 100.
Total length of bone in straight line	111	94	118
Height of proximal end	32.5	25	130
Width of proximal end	21	19	110
Side-to-side diameter at middle of length	16	12	133
Vertical diameter at middle of length	16	13.5	118
Side-to-side diameter above lower articular surface	23	19	121

The distal end of the fifth metacarpal is broken off, as well as a part of the proximal articulation. The total length of the fragment is 82 mm. The greatest width of the proximal end is 27 mm.; of that of the lioness, 22 mm.

The calcaneum is slightly injured at both ends. The following are the measurements of this calcaneum and that of the lioness:

Measurements of calcanea in millimeters.

Dimensions Taken.	Fossil felid.	*Felis leo.*	Ratio of fossil to *Felis leo* taken as 100.
Total length of bone on outer face	123	90	137
Height of surface for cuboid	31	22	141
Greatest height at surface for astragalus	50	39	128

The calcaneum of another lion is 106 mm. long.

The fourth metatarsal lacks the distal end. The greatest depth of the proximal end is 25 mm.; of that of the lioness, 21.5 mm.

The complete first phalange mentioned is 52 mm. long, measured on the upper surface and at the middle of the width. That of the third digit of the hind foot of the lioness is 41 mm. That of the fossil has the lower face flat or concave from side to side; in the lioness this face is convex from side to side.

DESCRIPTION OF PLATES.

PLATE I.

Figs. 1-3. *Testudo equicomes.* Parts of plastron. Type. × 1. *1*, Right epiplastron, seen from below. *2*, Right epiplastron, showing median suture. *3*, Left hyoplastron, seen from below.

Figs. 4, 5. *Mylodon harlani.* Teeth. × 2-3. *4*, Left front upper tooth, seen from right, or inner, side. *5*, Right first lower tooth, seen from behind.

Figs. 6, 7. *Hipparion cragini.* Two views of a tooth of the right side. Type. × 1. *6*, Tooth seen from right, or outer, side. *7*, View of the grinding surface.

Fig. 8. *Camelops huerfanensis.* Upper left milk-molar, seen from left, or outer, side. × 2-3.

PLATE II.

Fig. 1. *Equus complicatus.* Lower left cheek-teeth, pm_3, pm_4 and m_1.

Figs. 2-4. *Equus leidyi.* Upper jaw and teeth. × 2-3. *2*, Left jaw and four teeth, seen from below. *3*, First molar, seen from left, or outer, side. *4*, First molar, seen from in front.

Figs. 5, 6. *Camelops huerfanensis.* Teeth. × 2-3. *5*, Lower right second molar, seen from right, or outer, side. *6*, Lower right second milk-molar, seen from left, or inner, side.

PLATE III.

FIG. 1. *Testudo equicomes.* Plan of front of plastron of type. × 1-3.

FIGS. 2-4. *Mylodon harlani.* Sections of teeth. × 1. *2*, Section of first upper tooth. *3*, Section of third upper tooth. *4*, Section of first lower tooth. In the case of figures 2 and 4 the fronts of the teeth are above. In figs. 2 and 4 the inner faces of the teeth are toward the left; in figure 3 it is toward the right.

FIGS. 5, 6. *Equus leidyi.* Grinding surface of lower premolars and molars, × 1. *5*, Left premolars. *6*, Right molars.

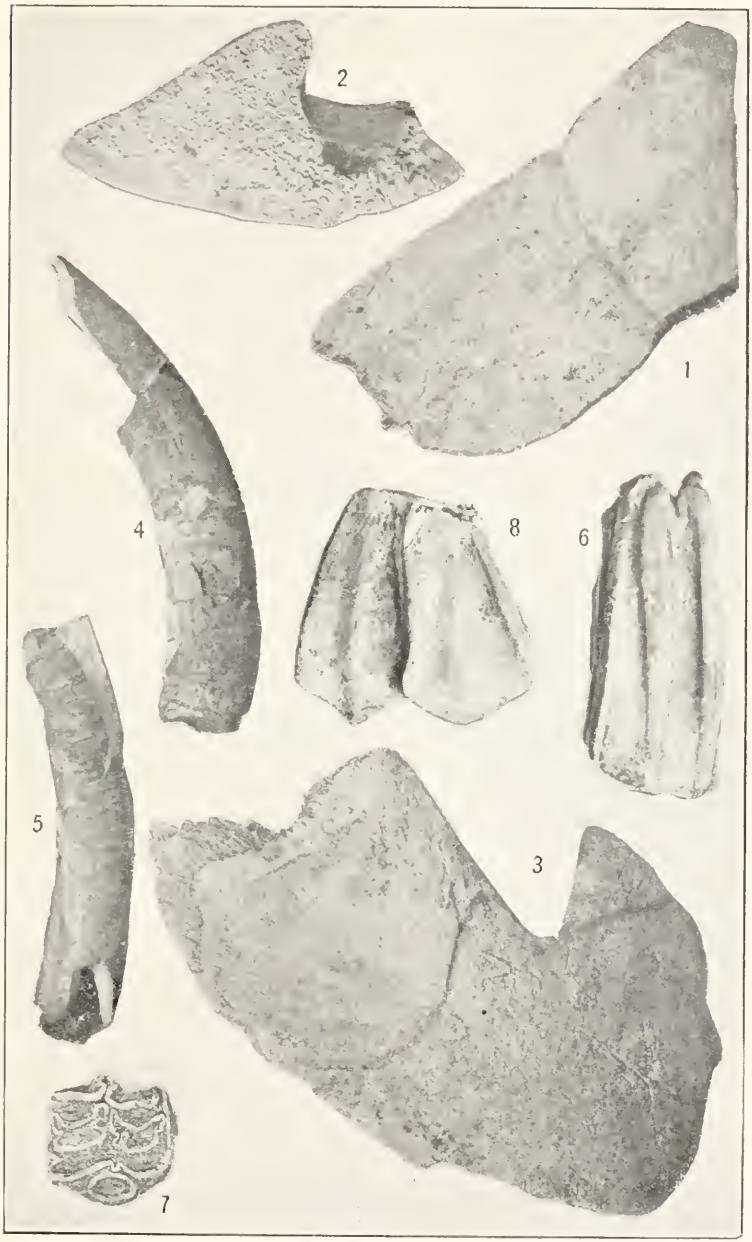
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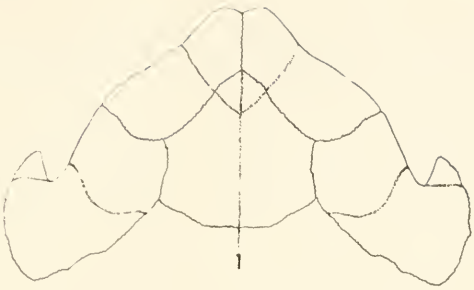
PLATE II.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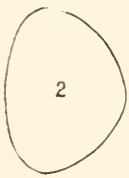
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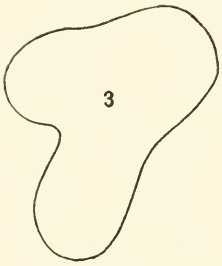
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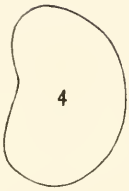
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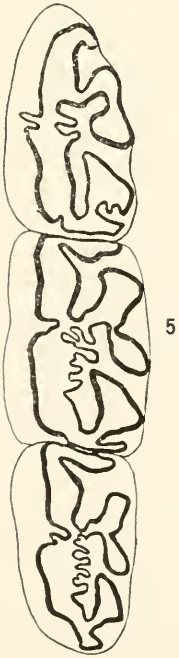
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THE

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(Whole Series, Vol. XX, No. 5.)

CONTENTS:

OGMODIRUS MARTINII, A NEW PLESIOSAUR FROM THE CRETACEOUS OF KANSAS. . . . . *S. W. Williston and Roy L. Moodie*

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THE KANSAS UNIVERSITY SCIENCE BULLETIN.

VOL. X, NO. 5.] JANUARY, 1917. [WHOLE SERIES VOL. XX, NO. 5.

Ogmodirus martinii, a New Plesiosaur from the Cretaceous of Kansas.

BY S. W. WILLISTON,

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ROY L. MOODIE,

Department of Anatomy, University of Illinois, Chicago.

THERE have been recorded from the Cretaceous deposits of Kansas five genera and sixteen species of plesiosaurs, as follows:

From the Comanche (in the restricted sense of the term), two species referred provisionally to *Plesiosaurus;* from the Benton, two species of *Trinacromerum* (including the genotype), one of *Brachauchenius*, the genotype, and one of *Elasmosaurus;* from the Niobrara, five species of *Elasmosaurus*, two of *Polycotylus*, including the genotype, and one of *Trinacromerum;* from the Fort Pierre, the genotype of *Elasmosaurus*, and one species referred provisionally to *Plesiosaurus*. Of the above, doubtless all those referred to *Plesiosaurus* belong to other as yet unrecognized genera; and in much probability several of those referred to *Elasmosaurus* from the Benton and Niobrara will be found eventually to pertain to other genera. One of these, especially *E. ischiadicus* Will., quite surely does not belong in that genus and may belong in the present genus. It was originally described as a problematical species of *Polycotylus* because of its short cervical vertebræ, but later quite surely eliminated from that genus because of its short ischia. For the present it may find a location in the following genus.

The present genus, *Ogmodirus*, from the Cretaceous of Cloud county, Kansas, is of peculiar interest in presenting certain intermediate characters between the Elasmosauridæ and Plesiosauridæ, the two distinctive families of long-necked plesiosaurs. The two families differ, aside from the skull, especially in the

structure of the pectoral girdle. The characters of the genus *Elasmosaurus* have been given by the senior author[1] as follows:

"Symphysis of mandibles short; teeth markedly anisodont. Neck with seventy-six true cervical vertebræ and three pectorals, the centra increasing in length to the fifty-eighth, and then decreasing in length to the dorsals, thence nearly uniform in length throughout the dorsal region. Posterior cervicals and dorsals much wider than high, and wider than long; spines wide and not high; zygapophyses weak. Pectoral girdle with large scapulæ meeting each other in the middle line. No interclavicular foramen; coracoids broadly separated posterior to the interglenoid thickening, their posterior ends not much dilated. Cervical ribs single headed. Ischia short."

Of these characters the number of cervicals is doubtless a specific character; the species *E. serpentinus* has but sixty cervicals. The elongation of the posterior cervicals is undoubtedly a real generic character, and is common to various species. The approximation of the scapulæ and absence of the interclavicular foramen, and also, in much probability, of the interclavicle, may or may not be of family value. The European forms referred to this family do not have, so far as published, the broad separation of the coracoids posteriorly. It is very probable that, when the skulls have been more attentively compared, *Cryptocleidus* and its allies will be excluded from the family Elasmosauridæ.

That the present genus is distinct from *Elasmosaurus* is evident. Until its skull and pectoral girdle are known its position in the family is doubtful. The only other long-necked genus with which it can be compared is *Leurospondylus* Brown,[2] recently described. So far as the description and figures of that genus apply to the present material the two genera can not be distinguished.

The material upon which *Ogmodirus* is based is represented by the following parts: fifty-one consecutive cervical vertebræ, eighteen caudal vertebræ, humerus, femur, many carpal and phalangeal bones, the right ilium, a part of a pubis, and various fragments of ribs and neural spines. This material was collected in 1909 by Mr. C. Boyce in Cloud county, Kansas, associated with the remains of another plesiosaur, and presented by him to the University of Kansas. Its horizon is probably the Fort Hays limestone of the basal Niobrara, though possibly, but improbably, from the uppermost horizon of the Benton. The species has been named in honor of Mr. H. T. Martin, in appreciation of his long,

1. Williston, S. W. 1906. North American Plesiosaurs: *Elasmosaurus, Cimoliasaurus* and *Polycotylus*. Amer. Journ. Sci., 4th ser., vol. xxi, No. 123, March, p. 225.

2. Brown, Barnum. 1913. A New Plesiosaur, *Leurospondylus*, from the Edmonton Cretaceous of Alberta. Bull. Amer. Mus. Natl. Hist., vol. xxxii, art. xl, pp. 605-615, figs. 1-7. December.

faithful and intelligent work in the Cretaceous deposits of Kansas for the University of Kansas.

The genus *Ogmodirus* (ὄγμος drawn out in a straight line and δειρή neck) has been defined as follows:[3]

Cervical vertebræ short, almost as wide as long, gradually increasing in size. The length and breadth are proportionate, as is indicated in the following table of measurements:

		Length.	Width.
Cervical vertebra	1	0.020 m.	0.030 m.
Cervical vertebra	6	0.023 m.	0.032 m.
Cervical vertebra	12	0.030 m.	0.038 m.
Cervical vertebra	20	0.034 m.	0.044 m.
Cervical vertebra	30	0.041 m.	0.052 m.
Cervical vertebra	40	0.045 m.	0.067 m.
Cervical vertebra	45	0.045 m.	0.067 m.
Cervical vertebra	51	0.046 m.	0.072 m.

Since there is nothing present of either the atlas or axis, the first cervical vertebra preserved must be at least the third of the series, possibly a later one. At any rate it is the first of the series preserved. No cervical ribs are preserved complete, though there are fragments of them. The rib facettes are present throughout the cervical series. (Fig. 1, text.) For a member of the Elasmosauridæ the neck is not extraordinarily long, containing probably about sixty vertebræ, and measuring about 2 meters. The paddles are not expansive, but short and thick, with the metacarpals and phalanges short and heavy.

The fore limb (plate II) has a complete length of 0.600 m., of which 0.152 m. belongs to the propodium, 0.050 m. to the mesopodium, 0.070 m. to the metapodium, and about 0.300 m. to the digital portion. This is making no allowance for the cartilage, ligaments and integument, which doubtless adds several millimeters to the length as given.

Cervical vertebræ. These centra are, as usual, nearly amphiplatyan. On the visceral surface they are, throughout the series, perforated by a pair of vascular foramina (plate IV, fig. 5), which have a diameter of 2 mm. On the fifteenth vertebra preserved this opening measures 6 mm. There are two articular surfaces for the articulation of the ribs (plate I). These articular surfaces gradually increase in size toward the dorsal region. On the third cervical they possess a length of 11 mm. and a breadth of 8 mm.; on the fifteenth a length of 18 mm. and a breadth of 14 mm. Superficially there appears over all the cervical centra many

3. Williston, S. W., and Moodie, Roy L. 1913. A New Plesiosaurian Genus from the Cretaceous of Kansas. Bull. Geol. Soc. Amer., vol. 24, No. 1, pp. 120, 121. March.

FIG. 1. The cervical vertebræ of *Ogmodirus martinii* drawn from the ventral side. The series is incomplete at both ends.

punctiform vascular pits and canals (plate IV, fig. 5), doubtless indicating a rich periosteal blood supply. The articular facttes for the neural arches are egg shaped, possessing a length of 31 mm. and a breadth of 15 mm. of the fifteenth vertebra. Between the articular facettes for the neural arch lies the shallow, hourglass-shaped canal for the spinal cord, or rather the meningeal canal. In the center of the canal occur two egg-shaped vascular pits. The centra are slightly constricted in the middle of the bone, although the visceral portion is larger than the neural portion, owing to the presence of the costal articulations.

The articular surfaces for the *neural spines* are identical in the most anterior and in the most posterior. They are oval in shape, with the central point most depressed. Between the depressions for the neural spines superiorly there are two nutrient foramina corresponding in position and undoubtedly in function to the ventral structures of the same.

The diapophysis stands out moderately; vertically elongate, apparently connected by a narrow surface upward on the arch. The lower extremity of the articular surface, however, reaches pretty well down on the ventrum.

Transverse diameter of the centrum	84
Vertical diameter of the centrum	78
Length	45–48

The better of the two dorsals is deeply cupped, circular in outline, with rather sharp rims, rather deeply concave on the sides and below and circular in cross section through middle. Vascular foramen situated high up on the sides, above the middle.

Length below	55
Length above	51
Transverse diameter	100
Vertical diameter	92

Only a few much water-worn fragments of spinous processes are preserved (plate IV, fig. 3); of the neural arch nothing is at hand. One of the best-preserved neural spines is deeply scarred by the teeth of some predaceous Cretaceous fish. The neural spine possesses a height of 85 mm. and a breadth of 31 mm. Outwardly the spine is smooth, the apex roughened for a cartilaginous tip, apparently indicating an immature animal.

The neural spines are relatively heavy, broad, and laterally flattened. Only fragments of them are preserved, but one of these fragments consists of a nearly complete spine. The tip was capped by a large amount of cartilage, another indication of the youthful

character of the individual. The edges are roughened for muscular attachment and the sides are smooth. The zygapophyses are rounded and saddle-shaped.

Measurements of Cervical Vertebræ.

(No. 441, University of Kansas Museum of Natural History.)

Length of most anterior vertebra preserved	20 mm.
Width of most anterior vertebra preserved	28 mm.
Height of most anterior vertebra preserved	17 mm.
Median width of neural canal	5 mm.
Length of the most posterior vertebra preserved	45 mm.
Width of same	70 mm.
Height of same	43 mm.

Measurements of Neural Spine.

(Possibly associated with the cervical vertebræ.)

Height of single spine preserved	85 mm.
Dorsal width	30 mm.
Ventral width	36 mm.

Among the material brought in by Mr. Boyce there was a string of eighteen caudal vertebræ which are possibly to be associated in this species. We will at least place them there provisionally until such a time as further information will permit their exact identification. They consist of four pygals and fourteen caudals. The pygals differ in form from the most posterior caudals, but have essentially the structure exhibited by the cervicals, *i. e.*, short, heavy, depressed, with lateral rib articulations. The most anterior pygal differs from the cervicals in being shorter, with the neurocentral articular surfaces wider apart, and the rib articular surfaces larger, the reduction in size of the nutrient foramina and the more marked amphicœlous character of the centra. This will sufficiently characterize the pygals. The caudals differ from the pygals in a further reduction along these same lines.

Measurements of the Pygals and Caudals.

Length of most anterior pygal	31 mm.
Width of same	60 mm.
Height of same	36 mm.
Diameter of neural canal	15 mm.
Length of rib articular surfaces	20 mm.
Width of rib articular surfaces	18 mm.
Length of most posterior caudal	23 mm.
Width of same	54 mm.
Height of same	38 mm.
Diameter of neural canal	9 mm.

The neurocentral articular surfaces are divided into two portions, separated by the rib articular surface. The most anterior of the pygals indicates a sacral intumescence for the hind limbs. Just what its size in proportion to the brain may be would be an interesting matter. The intumescence extends through several of the vertebræ and indicates an active use of the limbs as propelling organs. The most posterior cervical preserved has no indication of such an intumescence, so we must conclude that the neck is still incompletely represented even with the fifty-one vertebræ, and may have included six or eight more. Certainly none of the vertebræ described above as cervical belong to the dorsal series.

Forelimb. There was enough material present, although badly scattered, which seemed to belong to a single member, to reconstruct (plate II) a paddle which has been referred to the left side. This limb has a total length of 0.600 m., of which 0.152 m. belongs to the humerus, 0.050 m. to the radius and ulna, 0.070 m. to the carpals, and perhaps 0.300 m. to the digital division, although the distal phalanges are lost. The breadth of the limb at the level of the radius is 0.150 m., at the carpus 0.130 m. and at the third phalangeal row 0.120 m.

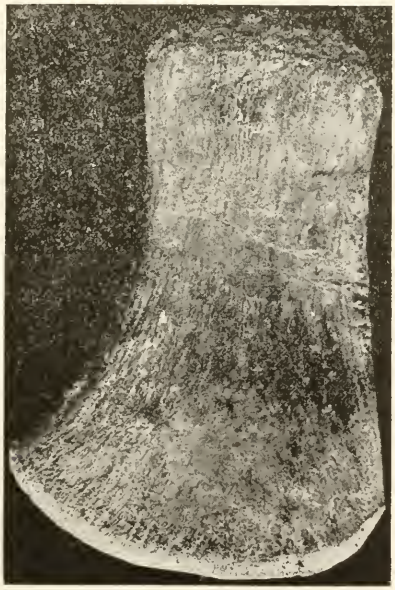


FIG. 2. The humerus of *Ogmodirus martinii* from the ventral side.

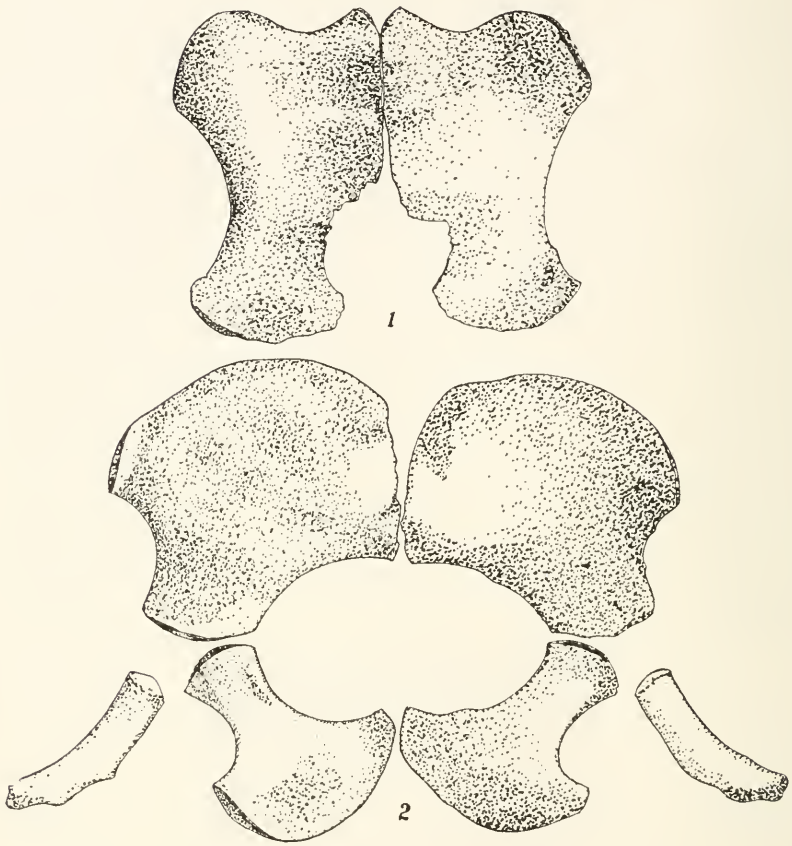


Fig. 3. (1) The coracoids of *Leurospondylus*. After Brown.
(2) The pelvic girdle of *Leurospondylus*. After Brown.
These figures are inserted here for the sake of completeness. The form described by Brown is very similar to *Ogmodirus*, and the parts of the skeleton which he figured and described are lacking in the present form.

The *humerus* is associated with the paddle remains provisionally. They were scattered and dissociated when the specimen was brought to the museum. It is possible that portions of two paddles are represented. The element is well characterized in the figure (plate III, fig. 1, and text fig. 2). It is short, heavy and thick, extremely so for a plesiosaur. Its lower surface is flattened and relatively smooth, with a slight tuberculation near the proximal end for muscular attachment. The ends are marked with pits and cones (plate III, figs. 2 and 4), resembling minature volcanoes. These pits and mounds doubtless indicate the fact of

emigration of the osteoblastic tissue from the medullary canal, which in this species is highly developed. The mounds correspond to the points of entrance of the *Canalis ossificans perforans,* which in mammals transmit the osteoblasts from the medullary portion of the diaphysis into the epiphysis. The element is regarded as a humerus simply on account of its shortness, since it has heretofore been an invariable rule among the plesiosaurs that the shorter propodial is the humerus.

The humerus is remarkably short and broad, thickened at the proximal end, so that at this point it shows a rounded outline in cross section. The element gradually expands distally, especially toward the median plane, so that finally the distal end appears partly separated by an indentation. On the upper surface the bone has been distinctly marked on the radial edge (plate III, fig. 1) by the teeth of some predaceous fish or reptile, inflicted shortly after the death of the animal. This is not a rare occurrence among Kansas Cretaceous fossils, and indicates that some of the Cretaceous vertebrates were carrion feeders.

There are no well-developed facettes on the ends of the humerus such as is so common among other plesiosaurian species.

Measurements of Humerus.

Length	0.152 m.
Width at proximal end	0.070 m.
Width at middle	0.071 m.
Width at distal end	0.112 m.
Thickness at proximal end	0.050 m.
Thickness at distal end	0.036 m.

The *radius* and *ulna* are flattened, rounded plates, with broad peripheral surfaces for articular cartilage. The ulna is much smaller than the radius and is more nearly rounded. The radius is elongate transversely, with the ventral surface concave and marked by several vascular pits. (Plate II.)

Measurements of Ulna.

Greatest breadth	47 mm.
Least breadth	42 mm.
Greatest thickness	21 mm.

Measurements of Radius.

Greatest breadth	70 mm.
Least breadth	52 mm.
Greatest thickness	25 mm.

The *carpals* (plate II) are of the same character as the radius and ulna. They can only be distinguished by size. The largest

of the carpals is regarded as the centrale. It is in the form of a rounded triangle. Its measurements are:

Greatest breadth	50 mm.
Least breadth	44 mm.
Thickness	20 mm.

The smallest carpal element is rounded, measuring 27 mm. in diameter.

The *phalanges* (plate II) vary in shape. There are twenty-three preserved. One or more of them may be modified carpals, but they are indistinguishable from phalanges. The broadest phalanx measures 29 mm. Its shaft is marked by a prominent pit which leads into a medullary cavity similar to that of the femur.

Breadth of the smallest carpal	0.028 m.
Breadth of the largest carpal	0.050 m.
Length of the largest carpal	0.041 m.

The first phalanx has a medullary cavity filled with calcite. From this cavity canals lead to the upper and lower surfaces, as in the humerus. The ends exhibit surfaces which were apparently covered with a richly vascular cartilage. It is quite evident that all of the carpal elements were embedded in a mass of cartilage and that possibly this cartilage included the ends of the proximal phalanges.

The phalanges of the second, third, fourth and fifth digits consist of cylindrical elements, with thickened ends.

Measurements of the Metacarpals and Phalanges.

Length of metacarpal I	0.033 m.
Length of phalanx I of digit I	0.032 m.
Length of metacarpal I	0.035 m.
Length of smallest phalanx in the first digit	0.020 m.

The *pelvic girdle* is represented by an incomplete pubis (plate IV, fig. 4) and an ilium belonging to the right side. The ilium is a slender, smooth, somewhat crushed bone, with expanded distal articular surfaces. The median portion of the ilium is somewhat constricted. At the proximal ends it is obliquely expanded for articulation with the sacral rib. The distal end is rounded, and is marked by numerous small vascular pits and canals. The ilium, like the humerus, is marked by the teeth of some Cretaceous fish.

Measurements of the Ilium.

Length	0.148 m.
Width at proximal end	0.018 m.
Width at distal end	0.054 m.
Length of the sacral rib articular fossa	0.043 m.
Width of the sacral rib articular fossa	0.028 m.

The portion of the pubis (plate IV, fig. 4) consists of the articular portion. It is marked, as are the other elements of the skeleton, by fine vascular pits and lines. The diameter of the articular end is 0.037 m.

Hind paddle. The left femur of the hind limb is preserved. It is distinguished from the humerus by its slender, nearly straight form (plate IV, fig. 1) and its almost circular cross-section at the proximal end. There are no sharply defined articular facettes. On the superior surface there is a roughened process for strong muscular attachment. Its position is such that this process can be regarded in a certain sense as a trochanter.

On the radial surface of the femur there is a distinct rounded foramen, similar to those described by the writers elsewhere. Since the bone is broken at the plane of this foramen, it shows that the foramen is the opening of an elongate rounded canal, which in turn leads into a large medullary cavity which sends spicular spaces into the surrounding bone. (Plate III, fig. 3.) This condition has been commented on several times, but no explanation has been offered to explain it. The structure has all of the appearance of the so-called "periosteal buds" so commonly figured in textbooks of microscopic anatomy.

Measurements of the Femur.

Length	0.175 m.
Diameter of the lateral foramen	0.006 m.
Diameter of the medullary cavity	0.015 m.
Length of the canal	0.020 m.
Length of the medullary cavity	0.025 m.
Length of one of the smaller canals	0.001 m.

In view of the possibility of identity, or at least a close relationship, between *Ogmodirus* and *Embaphias circulosus* Cope, we give herewith figures of the best of the type specimen of this genus and species (plate V) as it is now preserved in the Academy of Natural Sciences of Philadelphia. The type, collected by Mr. F. H. Charles from the Great Bend of the Missouri river, South Dakota, in 1893, consists of two dorsal and one cervical vertebra. The specimens present no generic characters and can not be again

recognized until more complete specimens from the same locality and horizon are studied. That they belong to a short-necked plesiosaur is probable, and this may be *Polycotylus* or something allied to it; though if the horizon is the Pierre Cretaceous, as is probable, the generic identity would be more doubtful. The greater concavity of the dorsal centra also indicates a distinct genus.

The cervical is flattened, nearly circular in outline, rather strongly concave; a single large vascular foramen below, but no ridge or fossæ, but very slightly concave from margin to margin. The articular rim encroaches much on the exterior.

The diapophyses stand out moderately; vertically elongate, connected by a narrow surface upward on the arch. The lower extremity of the articular surface, however, reaches pretty well down on the ventrum.

Transverse diameter of the centrum	84 mm.
Vertical diameter of the centrum	78 mm.
Length	45-48 mm.

The better of the two dorsals is deeply cupped, circular in outline, with rather sharp rims, rather deeply concave on the sides and below, and circular in cross-section through the middle; vascular foramen situated high up on sides, above the middle.

Length below	55 mm.
Length above	51 mm.
Transverse diameter	100 mm.
Vertical diameter	92 mm.

DESCRIPTION OF PLATES.

PLATE I.

The cervical vertebræ of *Ogmodirus martinii*, photographed from the ventral surface. $\frac{1}{3}$ natural size.

PLATE II.

The left fore paddle of *Ogmodirus martinii*, photographed from the dorsal surface. The elements are of doubtful association. $\frac{1}{3}$ natural size.

PLATE III.

Fig. 1. The right humerus of *Ogmodirus martinii*, photographed from the dorsal surface and showing at *S* the tooth marks of some predaceous fish. $\frac{1}{2}$ natural size.

Fig. 2. The distal end of the humerus, showing the pits and cones, resembling miniature volcanoes, the results doubtless of the exit of the *Canales ossificantes perforantes* carrying osteoblasts. $\frac{1}{2}$ natural size.

Fig. 3. A photograph of a broken section through the femur. This section shows, on the right, the canal leading into the calcite-filled cavity. These features are apparently growth characters.

Fig. 4. A photograph of the proximal end of the humerus to show at *A* the pits and cones.

PLATE IV.

FIG. 1. The left femur of *Ogmodirus martinii*. $\frac{1}{3}$ natural size.
FIG. 2. Neural spines.
FIG. 3. The right ilium.
FIG. 4. A portion of a pubis.
FIG. 5. Two views of a cervical vertebra.

PLATE V.

The vertebræ of the type of *Embaphias circulosus* Cope. Specimens in the Academy of Natural Sciences of Philadelphia: 1 and 2, lateral and end views of vertebra; 3 and 4, lateral and end views of other vertebra.

PLATE I.

PLATE II.

OGMODIRUS MARTINII.
Williston and Moodie.

PLATE III.

1

2

3

4

OGMODIRUS MARTINII.
Williston and Moodie.

PLATE IV.

1

3

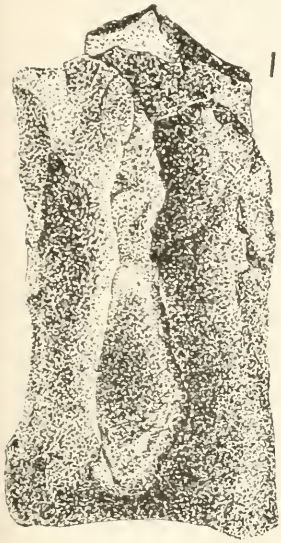
2

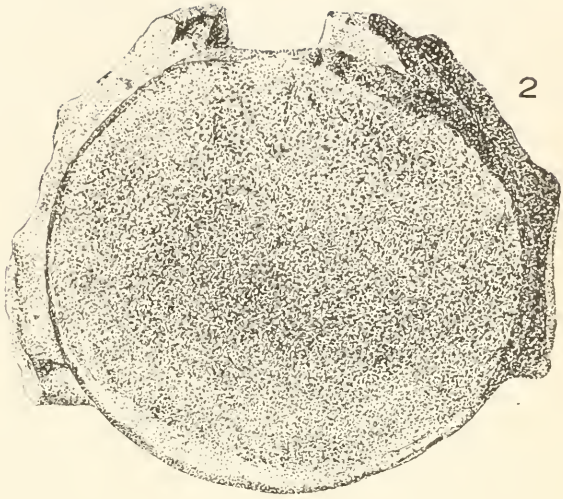
5

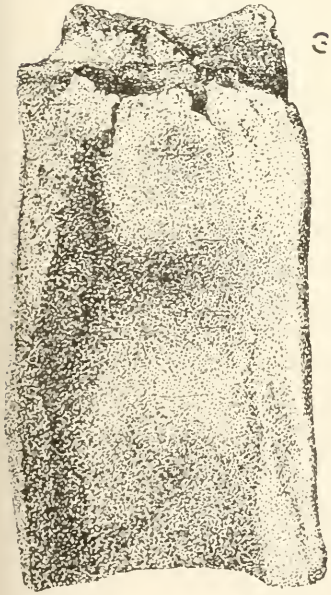
4

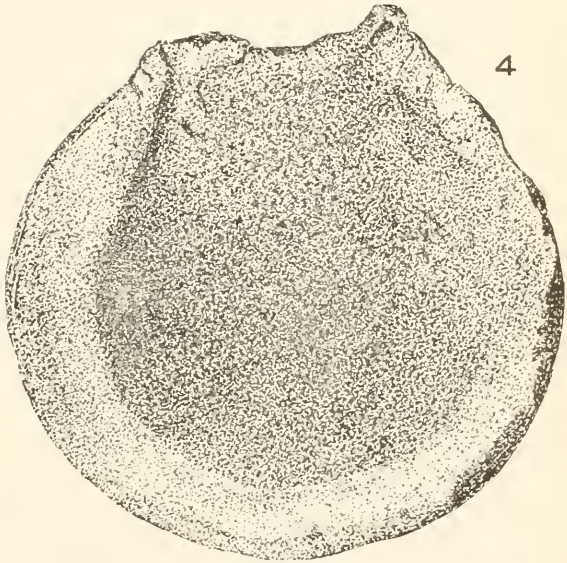
OGMODIRUS MARTINII.
Williston and Moodie.

PLATE V.









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CONTENTS:

AN ANATOMICAL STUDY OF CYCLOLOMA ATRIPLICIFOLIUM . . *Dudley J. Pratt.*

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An Anatomical Study of *Cycloloma atriplicifolium.*

BY DUDLEY J. PRATT.

A thesis submitted to the Department of Botany and the Faculty of the Graduate School in partial fulfillment of the requirements for the master's degree.

INTRODUCTION.

CYCLOLOMA ATRIPLICIFOLIUM is a coarse annual native to the central part of North America. The typical form of this hardy xerophyte is found in the Niobrara sand hills, according to the Phytogeography of Nebraska ('09). There the small, slender, moderately branched form found in other localities becomes a great bushlike type (5 dm. to 2 meters in diameter and 1 meter high), called the giant tumbleweed, which is well adapted to the tumbling habit because of its subglobose, compact top and innumerable seeds. The spreading tops and close aggregations of these tumbleweeds, sometimes several hectares in extent, crowd out other plant growth from the sandy plains or summits of the sand hills. On tilled soil, however, the smaller form of this weed is not hard to combat. The Iowa Geological Survey ('13) states the weed is easily exterminated in that state by cultivation.

There has been no anatomical work done on *Cycloloma*, recorded in the literature accessible to the writer, but there has been some ecological study of the genus, and of the species *atriplicifolium*. Bentham and Hooker ('80), and Engler and Prantl ('89), classify *Cycloloma* under the Chenopodieæ, and describe the genus. Solereder ('08) discusses the anatomical features of the Chenopodieæ, but does not include *Cycloloma* among the genera mentioned. Descriptions of both the genus and species are given by Britton and Brown ('13), Coulter ('09), and Gray ('08).

The plant studied was collected by Professor W. C. Stevens, of the University of Kansas, September 5, 1915, on dry upland

four miles southeast of Pratt, Kan. When gathered it was placed in a two per cent formalin solution. The specimen is ordinary in size, and is fresher than those commonly found in the early fall, due, no doubt, to the unusually frequent summer rains.

METHOD OF PROCEDURE.

The methods of anatomical study employed by Stevens ('11) were followed. Microchemical tests were made on sections cut free-hand with the razor. Microtome sections of all sizes of stem and root were made, and sections were made of five regions of stem and root imbedded in both paraffin and celloidin. Region I (fig. 29, *m''*) of the stem was cut within 5 mm. of a young branchlet tip; region II (fig. 29, *o''*) within 1 to 3 cm. of a young branchlet tip; region III (fig. 29, *h''*) from a branchlet of medium size; region IV (fig. 29, *r''*) from the base of a main branch; and region V (fig. 29, *s''*) from the main axis, 7 cm. above the root, where the diameter is approximately 1.5 cm. The sections of the five regions of root correspond in size to the five regions of stem. The general drawings mapping out tissue regions were made from material not imbedded, but the detail drawings were made for the most part from material imbedded in paraffin, but in a few instances in celloidin. All drawings were made with the use of the projectoscope.

THE STEM.

The main axis of the stem in my specimen is 15 cm. long; it varies in diameter from 2 cm. close to the root to 1 cm. where the last branches go off. From this main axis arise uniformly twelve erect branches, which are not straight but irregularly zigzag, and which are diffusely branched to fill out the globose contour of the plant. The bases of the two uppermost main branches unite to form the tip of the main axis of the stem. The branching habit of a main branchlet is shown in fig. 1. The stem has a glabrate surface, and a light green color with a purple tinge; but parts of the main axis and the large branches are colored a light brown. Due to a well-developed vascular system and collenchyma tissue of the ribs, even the branchlets are very resistant to breaking.

The zones of tissue for the five regions of stem are mapped out in figs. 2, 3, 4, 5 and 6; the detail of the tissue for the first four upper regions of stem is found in figs. 7, 8, 9, 10, and 11. The tissues of the five regions of stem, from the epidermis to the pith, inclusive, are described in the following paragraphs. Discussions

of the primary vascular bundles and of the woody cylinder of the stem follow.

The epidermis consists of one layer of cellulose-walled cells, the lumina of which appear rectangular or circular in cross section. The cell walls show more thickening down through the regions II and III (figs. 8, *a*, and 9, *a*); and the lumina of the cells in region IV (fig. 10, *a*) have become very small because of this thickening. The external wall, which is thicker than the other walls, does not have a cutinized layer in young and medium-sized stems, but develops a cuticle, which, although a very thin film in region I (fig. 7), is more prominent in regions II and III (figs. 8 and 9). The greatest thickness of the cuticle is only .002 mm., attained in region III (fig. 9). The cuticle stains a bright reddish-orange color in chloroiodide of zinc, and bright red in Sudan III and alcannin.

The epidermal cells of the ribs of the stem are elongated longitudinally (figs. 12 and 13) approximately four times their tangential dimensions (fig. 9, *a*), and show a slightly greater dimension tangentially than radially (fig. 9, *a*). The epidermal cells other than those on the ribs of the stem are slightly elongated longitudinally or have relatively equal longitudinal (figs. 14 and 15) and tangential (fig. 9, *a*) dimensions, and have greater tangential than radial dimensions (fig. 9, *a*). The inner tangential wall of the epidermal cells joins up in a somewhat irregular line with the primary cortex cells (fig. 10, *a*). In surface view *Salsoli kali* also has lengthened epidermal cells of the ribs and isodiametric epidermal cells between the ribs, according to Solereder ('08).

The epidermis of very young stems is thick.y covered with clothing and glandular hairs (figs. 16 and 17). As would be expected, the hairs are not so numerous in region II as in region I; still fewer hairs are found on region III, and usually only basal portions of hairs remain in region IV. The hairs of the stem are discussed later with the hairs of the leaf.

The stomata of the epidermis of the stem are like those described for the leaf in this article, and are almost as numerous, there being 117 per sq. mm. (Figs. 12 and 14.)

Subepidermal groups of collenchyma (fig. 9, *b*) projecting as ribs; groups of parenchyma (fig. 9, *c*) intervening between the ribs; also parenchyma (fig. 9, *i*) between the collenchyma groups and the starch sheath; and the starch sheath (fig. 9, *d*), make up the primary cortex.

The collenchyma cells in the outer part of the rib, then the cells situated farther in, are differentiated from the ground meristem by thickening of the cellulose walls as shown in regions I and II (figs. 7, *b*, and 8, *b*). The collenchyma, which is the sole strengthening tissue in region I, contains chloroplasts. In cross section the cells are usually as large as the epidermal cells or larger. A longitudinal view of the collenchyma cells of region III is shown in fig. 18. In region IV (fig. 10, *b*) we see the collenchyma has reached a greater development than higher up, and the lumina of the cells are smaller because of the thickening of the cell walls.

In most ages of stem seven groups of collenchyma, corresponding to the seven prominent ribs, are present. There are three main ribs in region I (fig. 2), giving this region of the stem a triangular shape. Ten ribs are present in region V (fig. 6).

The chlorophyllous tissue between the ribs is made up of ordinary parenchyma cells, the walls of which are thickly lined with chloroplasts (fig. 7, *c*). There are no intercellular spaces among these cells in region I (fig. 7, *c*); a few occur in the outer part of the chlorophyllous tissue of region II (fig. 8, *c*); and quite a number are present in region III (fig. 9, *c*).

On certain sides of the stem the cell walls of one to six rows of parenchyma cells, just exterior to the starch sheath, in region IV, (fig. 10, *o*) suberize their cell walls. These cells resemble the cells of the starch sheath in shape and size, and have thicker walls than the other parenchyma cells of the primary cortex. A phellogen layer arises in the parenchyma of the primary cortex on various sides of the stem in region V and below and forms cork (figs. 6, *b'*, and 19, *b'*) and lenticels (figs. 6, *a'*, and 20, *f'*). The epidermis and cells of the primary cortex exterior to the newly formed cork are sloughed off. According to Georghieff (*fide* Solereder, '08), the cork arises in the primary cortex in *Kochia* also, an allied genus. Solereder ('08) states that cork may be formed externally or internally in the Chenopodieæ; internally it is generally formed in the pericycle.

The starch sheath is well defined in all regions of stem by the shape and size of its cells, most of which have a long tangential dimension (fig. 9, *d*) and are lengthened longitudinally (fig. 21, *d*). The radial and the outer tangential walls of the starch sheath cells in portions of region V have become suberized.

The ground tissue of the pericycle has just been formed from the ground meristem in region I (fig. 7, *e* and *f*). In this region of stem two areas of the pericycle are distinguishable by the size of

their cells. The outer area of the pericycle is made up of small cells, which contain chloroplasts in very young stems (fig. 7, *e*), and the inner area of the pericycle is made up of much larger cells (fig. 7, *f*). There are three areas of pericycle in region II (fig. 8, *e'*, *e''* and *f*), and all the pericycle cells are larger in region II than in region I (fig. 7). The areas of pericycle *e'* and *e''* in fig. 8 correspond to the area of pericycle *e* in fig. 7. The cells in the outermost area of the pericycle (fig. 8, *e'*) have cellulose walls. In the areas of pericycle *e''* and *f* of fig. 8 the cell walls are lignified.

There is also an outer area of unlignified pericycle in regions III and IV (figs. 9, *e'*, and 10, *e'*). These cells are shown in longitudinal section of region III in fig. 21. The outer longitudinal strip of these cells, two cells deep radially, includes bast fibers. As seen in a cross section of region IV (fig. 10, *f'*) the bast fibers are arranged single or in short chains. This arrangement is found in nearly all the Chenopodieæ, according to Solereder ('08), who states, "it rarely consists of a closed sclerenchymatous ring, and is mostly composed of isolated groups of sclerenchymatous fibers." The occurrence of a sclerenchymatous pericycle is held to be an ordinal character in the Chenopodieæ by Georghieff (*fide* Solereder ('08).

A few bast fibers, irregularly arranged, also occur in three tangential rows of pericycle cells, just interior to the outermost row, in cross sections of region V (fig. 22, *f'*) and below. The bast fibers are approximately twice as broad as the wood fibers (figs. 23 and 24). The fibers stain a reddish-brown or a brilliant yellow in chloroiodide of zinc.

The lignification of cell walls in the areas of pericycle *e''* and *f* of fig. 9 in region III is more complete than in the corresponding areas of pericycle in region II (fig. 8). The cells of the area of pericycle *e''* of region III are shown in longitudinal section in fig. 25. In certain cross sections of region IV the two outer rows of cells of the central area of pericycle (fig. 11, *e''*) have thick lignified walls, and the outer cells in this area of pericycle have cellulose walls. The cell walls in the innermost area of pericycle are cellulose (fig. 11, *f*).

Generally all the cell walls in pericycle areas *e''* and *f* in fig. 11 of region IV are cellulose, or are very slightly lignified. The cell walls of the corresponding areas of pericycle in all regions of the stem below region IV are cellulose, or are very slightly lignified. (See the areas of pericycle of fig. 26, *e''* and *f*, in region V.) The

cells included in these areas of pericycle resemble pith cells, in shape and nature of cell walls.

The cell walls of the primary medullary rays are cellulose upward from a little above region II, as shown in fig. 7, *g*, of region I, and remain cellulose in certain sections of region IV (fig. 11, *g*). The cell walls of all but a few of the innermost cells of the primary medullary rays are lignified in regions II and III (figs. 8, *g*, and 9, *g*), and in some sections of region IV. The primary medullary ray cells, the walls of which are delicately pitted, are shown in longitudinal sections of region III in fig. 27.

The lignification of the cell walls in the pericycle areas *e''* and *f* (fig. 9) and the primary medullary rays in regions of stem from region IV up to a little above region II would seem to give strength and elasticity to these regions of stem. Probably the anomalous structure which forms a greater part of the woody cylinder of the stem in region IV and below (as described later) strengthens these regions of the stem sufficiently to make unnecessary the lignification of the cell walls of the pericycle areas *e''* and *f* and the primary medullary rays oftentimes in sections of region IV, and in all sections of regions below.

The pith cells are shown in longitudinal view in fig. 28. The cells of the pith do not break down to form hollow spaces, but persist in older sections of the stem. Generally the cell walls of the pith stain a deep purple color in chloroiodide of zinc, but also stain a delicate red with phloroglucin.

THE PRIMARY VASCULAR BUNDLES.

The primary vascular bundles are mapped out for region I in fig. 2; region II, fig. 3; region III, fig. 4; and region IV, fig. 5. Usually one or two of the primary vascular bundles of larger size are situated opposite each of the main ribs of the stem in regions I, II, and III. The course of the primary vascular bundles in the stem is mapped out in fig. 29, *j*. In regions above region IV the leaves remain on the stem, and the leaf traces traverse a portion of the anomalous structure (fig. 29, *t''*). The course of the leaf-trace bundles out of the stem up through the petiole into the leaf is discussed later. The leaf traces are broken apart in region V and below (fig. 29, *u''*) by further development of the anomalous structure.

The primary vascular bundles are differentiated into the phloëm and xylem elements in region I (fig. 30, *h'* and *j'*). In cross section some of the cells of the phloëm elements have walls unusually

thick for this region. This feature is discussed later. The central strip of procambium and the young water tubes of the primary xylem can be clearly discerned (fig. 29, *i'* and *a'''*). Immediately down the stem from region I the leaf-trace bundles appear in the pericycle (fig. 31, *k*), where they are crossing out into the leaf, and the cambial layer of the primary vascular bundles is discernible (fig. 31, *n'*). Some secondary phloëm and xylem have been formed by the cambial layer in region II (fig. 32, *m'* and *o'*).

In longitudinal sections of region III the sieve tubes, the companion cells and the phloëm parenchyma cells are discernible, as shown in figs. 33 and 34, *b'''* and *c'''*. In cross section the sieve plates are difficult to distinguish, their pits being very small (fig. 34, *d'''* and *f'''*). Certain sieve tubes have thickened their cell walls (fig. 34, *d'''*) and others have not (fig. 34, *f'''*). The cambium is active yet in a few of the bundles. Spiral and annular tracheæ of primary xylem are shown in figs. 36, 37 and 38. The parenchyma of primary xylem does not lignify.

In the secondary xylem of the primary vascular bundles the tracheæ have circular lumina, and are prominent. The tracheæ are pitted, having slightly elongated, bordered pits (fig. 39), or are of reticulate type (fig. 40). Solereder ('08) states, the tracheæ in the Chenopodieæ usually have simple pits, but mentions the genus *Axyris* as an exception. Georghieff (*fide* Solereder, '08) records scarlariform perforations with oblique, almost longitudinal, bars in *Axyris amarantoides*. At intervals portions of the cross walls of the elements of the tracheæ remain (fig. 39). The wood parenchyma cells of the secondary xylem of the primary vascular bundles are ordinary in size and shape, and have few and only inconspicuous pits (fig. 41). Wood fibers were not found to occur in the xylem of the primary vascular bundles. The fiber tracheids, which are rather few, vary in size and shape, and have bordered pits (fig. 42).

In the primary vascular bundles of region IV (fig. 43, *m'* and *o'*) more secondary phloëm and xylem are present than in the corresponding bundles of region III. The wood parenchyma of the secondary xylem of these bundles in this region of stem is well lignified.

A leaf-trace bundle with an active cambial layer is shown in fig. 31, *k*, and an older leaf-trace bundle in region IV is shown in fig. 44. The leaf-trace bundles do not grow to be very large. The cambial layer in most of the leaf-trace bundles of region III has ceased its activity. Solereder makes the following statement con-

cerning the development of the leaf-trace bundles in the Chenopodieæ: "The leaf-trace bundles sometimes possess considerable growth in thickness, and thus delay the appearance of the anomalous growth (*Camphorosma* and *Echinopsilon*, as also *Blitum virgatum*, *Chenopodium murale* and *C. hybridum*, according to De Bary, *Kochia prostrata* according to Georghieff.)"

As seen in some cross sections of region IV, and regions farther down the stem, the primary vascular bundles are very irregularly arranged, are more numerous than in regions higher up, and have the appearance of being imbedded in the pith. (See figs. 5, *j*, and 6, *j*.) This appearance is due to the fact that the cells in the areas of primary pericycle *e''* and *g* of fig. 26 and the cells of the primary medulary rays (fig. 44, *g*) in these regions of stem resemble pith cells in shape, and appearance of cell walls, which have remained cellulose or are very slightly lignified.

A few cells of the area of pericycle, *e''*, which have cellulose walls, are shown in longitudinal section of region IV in fig. 46. In regions of stem from region IV on down, parts of the anomalous xylem, which is first formed exterior to the primary vascular bundles by anomalous growth, resemble pith. This is shown for region V in fig. 26. A discussion of the origin and nature of the anomalous tissue appears later in this article.

The following statement quoted from Solereder ('08) throws light upon the pith-like appearance of the primary pericycle and primary medullary rays in most sections of region IV and all sections of regions below: "In the Nyctagineæ, Amarantaceæ and Chenopodiaceæ the ground tissue situated between the primary vascular bundles and the tissue formed at the commencement of the activity of the secondary meristem is occasionally differentiated like a pith, and in such cases the primary vascular bundles appear as apparent medullary bundles." Solereder also states that the vascular bundles, which he terms medullary bundles, appear in certain members of the Chenopodieæ, and adds that their development in this order shows that these bundles are rarely true medullary bundles, but are the normal leaf-trace bundles. According to Georghieff (*fide* Solereder), true medullary bundles occur in *Acroglochin persicarioides* Moq.

Evidently the primary vascular bundles of *Cycloloma atriplicifolium* have the same appearance in region IV and regions on down the stem as have the bundles which Solereder terms apparent medullary vascular bundles.

THE WOODY CYLINDER.

In region II there is a beginning of the formation of a woody cylinder, consisting of xylem groups of primary vascular bundles (fig. 3, *j*), and lignified pericycle (fig. 3, *e''* and *f*) and primary medullary rays (fig. 3, *g*), and holding imbedded the strands of phloëm belonging to the vascular bundles (fig. 3, *j*). The woody cylinder of region II is mapped out in fig. 3, and the detail of the tissue of the woody cylinder in a portion of a cross section of region II is shown in fig. 8. The woody cylinder in region III, mapped out in fig. 4, is larger than in region II, due to the larger size of the cells of the areas, *e''* and *f*, of the primary pericycle (fig. 9) and primary medullary rays (fig. 9, *g*) included, and to the addition of anomalous xylem (fig. 4, *m*), formed by anomalous growth.

The anomalous growth consists in the formation of one or several broad zones of xylem around the stem, and a narrow, broken zone of tissue (made up of phloëm groups, secondary medullary rays, and from one to several rows of pericycle cells exterior to the phloëm groups and secondary medullary rays), having thin cell walls and located just exterior to each xylem zone, both kinds of zones being formed by broken rings of arcs of secondary meristem, which arises successively around the stem in the pericycle. The arcs in each successive ring of arcs of secondary meristem are active for a time, but cease their activity before the next younger ring of arcs arises.

For convenience in presentation of the anomalous structure, frequently in this article the broad xylem zones are designated as xylem zones, and the narrow, broken zones having thin cell walls, as phloëm zones.

The number of cells in the outer area of pericycle is considerably greater in region III (figs. 4, *e'*, and 9, *e'*) than in region II (figs. 3 and 8). On various sides of the stem, arcs of the inner rows of the cells in this area of pericycle have just produced a secondary meristem in region III (fig. 47, *n*), and other secondary arcs, having arisen earlier on other sides of the stem, are in different stages of progress (figs. 48, *n*, and 49, *n*). Anomalous xylem has been formed on some sides of the stem in region III by the secondary arcs of meristem, as shown in figs. 4, *m*, and 29, *p''*, and 48, *m*. On some sides of the stem the secondary arcs have not yet arisen, as shown in fig. 9, *e'*.

In regions of stem just below region III, the arcs latest to be differentiated in the first ring of secondary arcs have arisen; and

a xylem zone around the stem (figs. 29, q'', and 50, p) and a phloëm zone located just exterior to the xylem zone (figs. 29, q'', and 50, q) have been formed in regions of stem a little farther down. The outer and inner borders of the two zones are very irregular in boundary (figs. 29, q, and 50, p and q), due to the varied activity of the first ring of secondary meristematic arcs, which is described in the following paragraph.

As seen in cross section, the formation of the xylem zone, and the narrow zone just exterior to the xylem zone, is as follows: Various arcs of the secondary meristem form radial strips of xylem on their inner side, and small, isolated groups of phloëm on their outer side (fig. 51, n''); some secondary arcs form radially longer strips of xylem on their inner side and do not form phloëm on their outer side (fig. 51, o''); and other secondary arcs form radial strips of xylem, and secondary medullary ray tissue between the phloëm groups (fig. 51, p''). What else can this area of tissue (figs. 50, r, and 51, r) located between the groups of secondary phloëm be called other than a secondary medullary ray? The xylem-phloëm zone is mapped out in fig. 50, p and q.

The outer two rows of cells of the phloëm zone, shown in figs. 50, t, and 51, t, are pericycle cells; the rest of the narrow zone is made up of the phloëm groups (figs. 50, s, and 51, s) and the secondary medullary rays (figs. 50, r, and 51, r); and the phloëm zone is interrupted by certain radial, anastomosing strips of xylem (figs. 50, u, and 51, u).

As seen in cross section, the newly formed anomalous xylem zone (figs. 50, p, and 51, p) coalesces with the outer part of the lignified primary pericycle (figs. 50, e'' and 51, e''). The anomalous xylem stains a light yellow color in chloroiodide of zinc (figs. 50, p, and 51, p); the part of the woody cylinder formed from lignified pericycle and primary medullary rays, a yellowish-brown (figs. 50, e'', f, and g, and 51, e'', f, and g). The anomalous xylem and the primary tissue included in the woody cylinder stain uniformly with phloroglucin.

In region IV (figs. 5, and 29, r''; and 10) more xylem zones, and the phloëm zones alternating with the xylem zones in a radial direction, have been formed by the successive rings of arcs of secondary meristem, arising in the pericycle. The arcs in the rings of secondary meristematic arcs, arising later than the first ring of secondary arcs, do not have their origin directly exterior to the phloëm groups previously formed by the next older ring of secondary arcs, as illustrated in fig. 48, n, where a secondary arc

has just arisen. A similar arc, forming the fourth ring of xylem in region IV, is shown in fig. 52, *n*. The arcs, in a ring of secondary arcs of one region of stem, anastomose both tangentially and radially with the arcs, in the corresponding ring of secondary arcs, in the region of stem below (figs. 5, *n*, and 29, *n*).

The number of xylem-phloëm zones on the various sides of the stem may differ, ranging from two to four in region IV (fig. 5); from five to eight in region V (fig. 6); and from eight to twelve in the part of the main axis having the greatest diameter.

The xylem groups of the primary vascular bundles are not included in the woody cylinder in region IV; for only the outer part of the primary pericycle, on various sides of this region of stem, lignifies completely enough to be included as a part of the woody cylinder (figs. 5, *e''*, and 11, *e''*), and the rest of the woody cylinder in region IV is anomalous tissue, composed of the xylem zones holding imbedded the phloëm zones, as shown in figs. 5, and 29, *r''*. On other sides of the stem, in region IV, all the cells of the primary pericycle (fig. 5, *e'*, *e''*, and *f*) and primary medullary rays (fig. 5, *g*) have cellulose walls, and are not included in the woody cylinder. In all regions of stem below region IV, none of the cell walls of the primary pericycle (fig. 25, *e'*, *e''* and *f*) and primary medullary rays (fig. 44, *g*) have lignified deeply enough to be included in the woody cylinder, and the woody cylinder in these regions of stem has been formed by anomalous growth only. (See figs. 6, and 29, *s''*.)

The relative radial breadth of the xylem-phloëm zones is shown in cross section in figs. 5 and 6. The anomalous zones are complete rings around the stem, or as segments of rings have an irregularly concentric arrangement in cross section, and undulate slightly in a radial direction in their course around the stem (figs. 5 and 6). The xylem zones and the phloëm zones anastomose both tangentially and radially (figs. 5, *u*; 6, *u*; and 29, *u*).

The xylem zones are generally made up of the tracheæ, a few lignified parenchyma cells bordering the tracheæ, and the numerous wood fibers (fig. 51, *p*). The wood fibers are shown in longitudinal view in fig. 24. In some sections of region V and below, the tissue lying on the inner side of some of the xylem zones is made up entirely of wood fibers (fig. 53, *p''*); and the remainder of the xylem in each of these xylem zones (about 80 per cent of the entire zone) is composed of wood parenchyma and tracheæ (fig. 52, *q''*).

One or several inner rows of cells, or almost all the inner half, of the innermost anomalous xylem zone, on certain sides of stem in some cross sections of region V (fig. 26, *c'*), and in all cross sections of regions of stem below, is made up of thin-walled cells, which resemble pith cells in cross section in shape and relative size and have cellulose or slightly lignified walls (figs. 54 and 55). The rest of this zone is of the usual xylem structure (fig. 25, *d'*).

Spiral tracheæ are not present in the xylem of anomalous growth. The tracheæ are arranged in radial rows associated with other tracheæ scattered through each xylem zone (figs. 26 and 51), or have a very irregular arrangement (figs. 50 and 53). The lumina of the tracheæ are usually circular in shape, but some are oval. The lumina of the tracheæ range from .010 to .080 mm. in diameter, being relatively quite large, when compared with the lumina of the tracheæ in other members of the Chenopodieæ. Solereder ('08) states the usual diameter is .015 to .045 mm. in the Chenopodieæ.

The lumina of the tracheæ are usually larger in the xylem zones (fig. 53, *p*) external to the innermost xylem zone (fig. 51, *p*), but the number of tracheæ and the sizes of the lumina of the tracheæ are the same for relative areas of each of the xylem zones exterior to the innermost xylem zone.

In cross sections the wood fibers appear irregular in shape and arrangement (fig. 53, *o''*), or may be rectangular or almost square and arranged regularly in radial rows (fig. 57).

The innermost anomalous phloëm zone has been described above. The other phloëm zones resemble the innermost phloëm zone, but are somewhat broader, being composed of larger groups of phloëm (fig. 57, *s*), larger regions of secondary medullary ray tissue (fig. 57, *r*), and two or more rows of pericycle cells located exterior to the phloëm groups and secondary medullary rays (fig. 57, *t*). The anomalous phloëm, although the cells are larger, resembles the phloëm of the primary vascular bundles (fig. 57, *s*). The cell walls of most of the secondary medullary rays lignify to some extent, but are thin (fig. 57, *t*). A few cells of a secondary medullary ray, the walls of which are cellulose, are shown in longitudinal section in fig. 59. The pericycle cells, in each phloëm zone, located exterior to the phloëm groups and secondary medullary rays (fig. 58, *t*) are similar to the cells of the secondary medullary rays in size, shape and nature of cell walls. A longitudinal view of these pericycle cells is shown in fig. 60.

As seen in cross section (figs. 5, *q*, and 6, *q*), the three inner phloëm zones are interrupted by numerous radial anastomosing strips of xylem (figs. 5, *u*, and 6, *u*). The other phloëm zones are traversed by a much lesser number of these (fig. 6). The radial strips of xylem are usually made up of wood fibers, as shown in fig. 10, *u*, although some contain tracheæ, as shown in fig. 56, *u*. The peculiar location of these radial anastomosing strips of xylem is due to the varied activity and anastomosis of the arcs of secondary meristem by which the radial anastomosing strips of xylem were formed. The anastomosis of the arcs of secondary meristem is described above. The phloëm zones in region V and below are joined up in cross section by radial anastomosing strips of tissue having cells which resemble the secondary medullary ray cells (figs. 6, *g'*, and 58, *g'*).

As seen in cross section, in the anomalous zones there are radial rows of cells having a radial dimension from two to four times as great as their tangential dimension (fig. 58, *g'''*). These radial rows of cells are narrow, being only one to four cells broad tangentially. None of these radial rows of cells in the sections examined extend entirely from the outer to the inner zone of anomalous growth, but generally traverse only a few zones. In the phloëm zones these cells are pericycle cells (fig. 58, *h'''*) and secondary medullary ray cells (fig. 58, *i'''*). In the xylem zones these cells resemble wood parenchyma in size and proportion, and have thick, slightly lignified walls (fig. 57, *j'''*).

As far as the writer has been able to ascertain, no use made of the anomalous structure by the plant has been mentioned in the literature available.

I am of the opinion that the anomalous xylem is an ample strengthening tissue, and serves principally for water storage, not so much for conduction. Probably the xylem of the primary vascular bundles conducts sufficiently for the transpiration of the leaves. The leaf-trace bundles traverse the two innermost anomalous xylem-phloëm zones in region IV (fig. 29, *t''*). Below region IV the leaf-trace bundles are broken apart by further anomalous growth (fig. 29, *u''*), and the leaves do not remain on this region of stem. Evidently from region IV on down the stem all the anomalous xylem zones are available for water storage.

The presence of many phloëm strands in the anomalous structure is quite striking, for it seems there is enough phloëm in the primary vascular bundles to conduct the photosynthetic products of the leaves. But there is much photosynthetic tissue in

the primary cortex in all regions of stem above region V, and on certain sides of the stem in regions below, on sides where the parenchyma has not been destroyed by the formation of cork within, and no doubt so much anomalous phloëm is necessary to store and to transfer the food, made in the photosynthetic tissue of the stem, for use in the rapid formation of the many anomalous xylem-phloëm zones. The photosynthetic products can be transferred readily from the parenchyma of the primary cortex through the starch sheath and pericycle to the anomalous phloëm. The products of photosynthesis may also travel from one anomalous zone to another because of the anastomosis of these phloëm zones (fig. 29, *g'*), and finally to the phloëm of the primary vascular strands, whence presumably it would go to the nutrition of the seeds later in the season.

The anomalous structure found in the Chenopodieæ occurs in all the Chenopodiaceæ having considerable growth in thickness, and is found in the Nyctagineæ and the Amarantaceæ, according to Solereder ('08), in his review of the anatomical features of Chenopodieæ. He quotes from Morot the following statement regarding the course of the development of the anomaly: "Secondary rings or arcs of meristem (the latter anastomosing reticulately) arise in centrifugal succession in the pericycle (internally to the bast fibers, where these are present) and produce secondary vascular bundles as well as conjunctive tissue of varying structure. The xylem portions of these secondary vascular bundles always arise on the inner, the bast portions on the outer side of the secondary meristem."

According to Morot (*fide* Solereder, '08), the appearance of the anomaly in transverse sections of the stem varies greatly between two main types, relatively to the nature of the meristem, and of the tissue intervening laterally between the bundles in each ring of secondary bundles. In the first type the broad, woody rings alternate in the radial direction with rings of tissue having thin cell walls; the woody zones are traversed by medullary rays of varying breadth, the cells of which have thin or lignified cell walls. The rings of tissue with thin cell walls consist of phloëm portions of the vascular bundles associated with variable amounts of a tissue, which Morot terms "parenchymatous conjunctive tissue." In the second type, which occurs more commonly than other types, the vascular bundles are arranged concentrically, spirally, or irregularly, and the bundles are imbedded in tissue, which Morot terms "prosenchymatous interfascicular tissue."

The phloëm groups, which vary in size and are sometimes very small, are associated with lignified or unlignified parenchymatous tissue.

The types of anomalous structure found in the Chenopodiaceæ, Amarantaceæ, Nyctaginaceæ and Tetragonieæ, and in species of *Mesembryanthemum* and *Phytolacca*, are not adaptive anomalies, but merely cases of variation of design; for the abnormal features may be various in the several members of a family, and even in the different species of a single genus, and yet not be connected with the ecological relations of the plants concerned, according to Haberlandt ('14). He makes the following statement concerning the place of origin of the secondary arcs of meristem: "According to Morot, each new arc of cambium arises opposite a phloëm group and extends laterally on each side until it meets an older cambial layer. Herail, on the contrary, maintains that each new cambium begins to develop at one end as a lateral continuation of an antecedent cambial layer; then it extends gradually across the leptome, and sooner or later rejoins an older strip. Leisering believes that both possibilities may be realized."

Anomalies have been found in many lianes as well as in certain small shrubs and herbs, according to Haberlandt ('14), but Hill ('01) has examined an anomaly in *Dalbergia paniculata*, a tree native to south and central India. He states, the anomalous structure is "extremely interesting and equally surprising for a tree attaining the height of sixty feet." From a preliminary examination of the wood, Hill describes the appearance of the anomaly in cross section somewhat as follows: narrow zones of tissue (termed by Hill as "narrow zones of the nature of phloëm") and broad zones of woody tissue alternate in a radial direction. The anomalous zones are traversed by narrow medullary rays. The following statement in regard to the structure of the anomaly in the stem of *Dalbergia paniculata* are quoted from Hill: "By the examination of a transverse section it may at once be seen that the narrow, abnormal zones are of the nature of phloëm, which is accompanied by a certain amount of cambium. This cambium is situated on the side nearer the center of the stem and abuts directly on the xylem elements. . . . The wood is made up of the usual elements; that is to say, vessels, fibers, and parenchyma."

THE ROOT.

The root is a taproot, light brown in color, with approximately the same length and diameter as the main axis of the stem, and having only a few lateral roots attaining much size. Many very small rootlets, with suberized walls for several of their outer layers of cells, are retained throughout the main root. One to several of these arise from each of the numerous grooves in the root. The lateral roots and rootlets extend into the central xylem cylinder or into the first, second or third formed anomalous xylem zone. It has the same habits of growth in thickness as the stem.

The general areas of tissue for regions I, II, III, IV and V are mapped out in figs. 61, 62, 63, 64 and 65. The detail of tissues in regions I, II, III and IV can be found in figs. 66, 67, 68 and 69.

The resemblance in structure of the corresponding tissue of root and stem is so close it is not necessary to give a detailed description of the root here. In very young roots (fig. 66, *b'*) the two or more outer rows of cells become suberized to form cork. Apparently no cork cambium has arisen. Later a few layers of cells, located just interior to these two or more outer rows of primary cortex cells having suberized walls, suberize their cell walls. The cells of the starch sheath can not be clearly discerned, as in the stem, except in portions of certain sections. In figs. 61 and 66 are shown the central xylem cylinder, the phloëm groups of the primary vascular bundle, and the primary medullary rays. The water tubes of the central xylem cylinder are large, and stand out well when stained with phloroglucin (fig. 66, *m''*).

Rings of arcs of secondary meristem arise successively in the pericycle, as in the stem, and form anomalous xylem-phloëm zones. An arc of secondary meristem is shown in fig. 70. In region II (figs. 62 and 67) the anomalous growth has begun, and a second xylem-phloëm zone is starting to form in region III (fig. 63); and the fourth zone, in region IV (fig. 64). From eight to ten xylem-phloëm zones are present in region V (fig. 65); ten to fourteen in the region of the main root having the greatest diameter.

The zones of the anomalous structure are more nearly concentric and broader radially than in the stem. The anomalous zones anastomose both tangentially and radially, but the anastomosis of the first three xylem-phloëm zones is not so frequent as in the corresponding zones of the stem. The three innermost xylem-phloëm zones of the anomalous structure are more nearly concentric than in the stem (fig. 64). The number and arrangement

of tracheæ for unit areas in cross sections is the same as in the xylem zones exterior to the innermost anomalous xylem zone of the stem, but there are relatively more tracheæ having the larger diameter (fig. 65). Commonly the part of the xylem lying on the inner side of the zone is made up entirely of wood fibers (fig. 69, *m''*), and about 80 per cent of the xylem zone has the usual structure found in the xylem zones of the stem (fig. 69, *n''*). The walls of the pericycle cells included in each phloëm zone lignify slightly. The cell walls of the secondary medullary rays usually remain cellulose.

Traversing the anomalous zones in cross sections of the root, as in cross sections of the stem, are the radial rows of cells having a radial dimension from two to four times as great as their tangential dimension (figs. 65, *g'''*, and 69, *g'''*). In the zones of tissue designated as phloëm zones in this article these cells are pericycle cells and secondary medullary ray cells; in the xylem zones these cells resemble wood parenchyma in size and proportion. The walls of many of these cells are lignified, as are the walls of similar cells in the stem; but all these cells in certain sections of the root have cellulose walls, and in many regions of the root are in direct connection with the vascular elements of the very small rootlets, which extend from the exterior grooves into the central vascular cylinder or inner anomalous xylem zones of the main root. The absorbed water is readily transmitted by these cells, included in the radial strips, to all parts of this region of root.

THE LEAF.

The leaves of *Cycloloma atriplicifolium* are alternate and oblong-elliptic, with pointed lobes. Like the stem, the leaves are colored a light green, and are purplish tinged. The leaf is not unusual in structure, and possesses many of the leaf characteristics of the Chenopodieæ mentioned by Solereder ('08). The areas of tissue in a cross section of a typical leaf are mapped out in fig. 71.

The epidermal cells are much more irregular in shape, as seen in cross section, than in the stem (figs. 72, *a*, and 73, *a*). The cells of the upper and lower epidermis are shown in surface view in figs. 74 and 75, and the lengthened cells of the upper epidermis over the main rib are shown in fig. 76. The cell walls of the epidermis are cellulose, except the cuticle, which reaches a thickness of only .001 mm. Solereder ('08) states that in spite of the xerophilous character of many species of Chenopodieæ, the cuticle rarely attains a considerable thickness.

Many clothing hairs (fig. 77) and a few glandular hairs (fig. 78) can be found on the young leaves, but only a few hairs are present on older leaves. Most of the hairs are located at the edge of the leaf, but on young leaves quite a number are scattered over the upper and lower surfaces.

Each clothing hair is seated on a prominent basal cell, above which it consists of a chain of one or more short cells (fig. 77, *a''*), distinguishable by a very thin cuticle from a long terminal portion of one or several cells (fig. 77, *u'*) having thin cellulose walls without cuticle.

The glandular hair has a basal portion made up of a chain of two or more cells with a very thin cuticle (fig. 78, *a''*), and a terminal portion consisting of a row of three or more cells, usually all but the end cell being conspicuous on account of cell contents and a thin cuticle. Cell contents are present in the large spherical or ovoid terminal cell of certain of these hairs (fig. 78, *b'*). The wall of this terminal cell stains blue in chloroiodide of zinc, and does not stain appreciably in Phloroglucin or Sudan III. The contents of the hair cells is discussed in the section on the nature of cell contents.

There are two types of hairs on the stem similar to the hairs on the leaf. A clothing hair of the stem is shown in fig. 16, and a glandular hair is shown in fig. 17.

The stomata are of ordinary type (figs. 74, 75 and 76). Solereder ('08) gives the absence of a definite type of stoma as a noteworthy feature in the leaf of the Chenopodieæ. The cuticle extends over the guard cells (fig. 73, *f''*). The stomata are almost as numerous in the upper epidermis as in the lower epidermis of the leaf, there being 122 stomata per sq. mm. in the upper epidermis, and 128 per sq. mm. in the lower. Usually the long axes of the stomata, as seen in surface view, are placed at an angle with the main rib of the leaf, although many are parallel with the main rib in both young and old leaves. The transverse arrangement of the stomata is not uncommonly shown by narrow leaves in the Chenopodieæ, but also occurs on the stem, according to Solereder ('08).

The mesophyll of the leaf is approximately 65 per cent photosynthetic tissue and 35 per cent water-storage tissue (figs. 71, *c* and *d*; 72, *c* and *d*; and 73, *c* and *d*). Solereder mentions the differentiation of the mesophyll into assimilatory and aqueous tissue as one of the leaf characters of the Chenopodiaceæ correlated with their dry-land habitat.

The water-storage tissue is made up of large cells centrally located and lying next the border parenchyma adjacent to the vascular elements (figs. 72, *d*; 73, *d*; and 79, *d*). The vascular elements of the leaf are imbedded in the mesophyll in closer proximity to the lower than the upper epidermis (figs. 71, *g*; 72, *g*; and 73, *g*). Under the upper epidermis of the leaf is a palisade layer of mesophyll, and just below there is a layer of mesophyll parenchyma joining up with the water-storage tissue (figs. 70, *l'''* and *m'''*, and 71, *l'''* and *m'''*). A surface view of a bleached leaf embracing the epidermal, the palisade and the vascular bundle systems is shown in fig. 80. Between the water-storage tissue and the lower epidermis is a layer of mesophyll two or three cells deep (figs. 71, *n'''* and 72, *n'''*). The intercellular spaces in this are too small and regular to form a typical spongy tissue. The mesophyll cells are shown in tangential section in fig. 81. A typical spongy tissue has not been observed in any species of the Chenopodieæ, according to Solereder.

In the main rib of the leaf collenchyma cells join up the water-storage tissue and cells of the lower and upper epidermis, as shown in figs. 71, *b*, and 72, *b*, and the main rib projects out farther on the under side of the leaf than on the upper, there being more collenchyma in the under side of the main rib. Collenchyma serves as a strengthening tissue in the tip of the leaf and also in the tip of each lobe of the leaf, as is shown in fig. 82.

The venation of the leaf is mapped out in fig. 83, which is made from a whole leaf bleached and cleared by the method of Peace ('11). To hasten the removal of the abundant calcium oxalate crystals, the leaves treated were left over night in 10 per cent hydrochloric acid after being treated with 5 per cent potassium hydroxide solution. The leaves were then stained with safranin in a concentrated chloral hydrate solution; and the surplus stain having been washed out in distilled water, the leaf was bleached in chloral hydrate solution until just the vascular system retained the red color. The leaf venation is very irregular and does not form a close network. In each lobe of the leaf a main branch of the vascular system finds its termination (fig. 83).

The venation of the leaf ends blindly throughout the mesophyll (fig. 84), except at the edge of the leaf, where the ultimate endings are groups of tracheids extending out close to the epidermis (fig. 85). The ultimate endings of the venation stand on an average of .9 mm. apart in the greater part of the mesophyll of the leaf, but the tracheid endings at the edge of the leaf stand

about .5 mm. apart. The tracheids are of three kinds, spiral, annular, and reticulate (figs. 84, p'', q'', and r''; and 85, p'', q'', and r'').

The xylem, phloëm and border parenchyma cells of a vascular bundle of the leaf are shown in cross section in fig. 72. The border parenchyma cells in leaves, which were left in 5 per cent potassium hydroxide solution for bleaching, are shown in tangential section in fig. 79. The pits in the end walls of these elongated cells are not conspicuous in unbleached sections (fig. 86).

The vascular system of the petiole is made up of separate vascular strands. This is shown in the cross section of a petiole from the base upward in figs. 87 to 89, inclusive, in which the five vascular strands are mapped out. The cross section of the petiole in fig. 87 was cut from the base of the petiole; fig. 88, midway between the base and tip of the petiole; and fig. 89, from the tip of the petiole. Each vascular strand has a sheath of border parenchyma. A cross section of two of the vascular bundles of the petiole is shown in fig. 90, *j*. Petit (*fide* Solereder, '08) has found that isolated vascular bundles form the vascular system of the petioles in species which he examined in the genera *Artiplex*, *Blitium*, and *Chenopodium*.

In the figs. 91 to 95, inclusive, there is mapped out a series of portions of the cross sections of the stem from below the petiole attachment to a little above. The five leaf-trace bundles are mapped out in fig. 91. In fig. 92 the leaf traces are crossing out of the stem into the petiole. Successive steps in the closing of the leaf gap are shown in figs. 93, 94 and 95.

NATURE OF THE CELL CONTENTS.

The Stem.

Distributed through the protoplasts of the cells of the epidermis, and of the collenchyma and parenchyma of the primary cortex, there are quite a number of large globules (and many small globules) which stain like oil with Sudan III and alcannin (fig. 9, q'). Similar globules are present in the cells of the phloëm of primary vascular bundles, and of the phloëm and secondary medullary rays of the anomalous tissue. The globules in newly cut sections of formalin material, left in Sudan III for twenty-four hours, stained red; in alcannin, pink. Some of the globules in sections which had remained in xylene, chloroform or ether for twenty-four hours, and were then left in either of the two stains mentioned above, stained as before, but nearly all the globules

had been dissolved out. All the globules which stain like oil dissolved out in sections left in xylene, chloroform or ether for forty-eight hours. Certain of these globules stain evenly, but most of them stain unevenly. Usually a spherical core (ranging from 4 to 20 per cent of the volume of the globule), located in the center or at the side of each unevenly stained globule, stains a darker or lighter red than the rest of the globule. Two separate spherical cores were present in each of certain globules observed.

Saponification tests on fresh sections did not indicate the presence of saponifiable oil in the globules. The Tunmann reagent used for the tests was made up fresh, and was tested on castor-bean sections, the oil of which saponified. The test was repeated on freshly cut sections of stem, and more reagent was made available to the mounted sections by placing small strips of cork at the edge of the cover-slips, which were then sealed with wax (65 per cent beeswax and 35 per cent rosin), or by the use of hollow-ground slides. The slides were placed in the electric oven and examined daily. The globules, which stain like oil, dissolved out in from twenty-four to forty-eight hours, and no saponification was evident in sections examined with the aid of the microscope or polarizer.

The oil globules in sections steamed for eight hours and then left in Sudan III or alcannin for twenty-four hours stained like the globules in sections, newly cut from formalin material, left in either of the two stains for twenty-four hours. Evidently no substance in the globules escapes when the sections are steamed, for the globules average the same size as the globules in sections not steamed. Condensed vapor from steam, which had been passed over sections and then condensed on cold petri dishes, did not stain yellow when exposed to the vapors from heated iodine crystals, and the presence of violatile oil was not demonstrated.

The above tests indicate that an oil is present in the globules, but evidently not a volatile nor a saponifying oil.

In many of the cells containing globules staining like oil there are also greenish-yellow globules, which do not dissolve out after the sections have remained seven days in xylene, chloroform or ether, and which do not stain when the sections are left in Sudan III or alcannin for forty-eight hours (fig. 9, *r'*). These globules were tested for glucosides as follows: Sections were boiled in 5 cc. of Fehling's solution, and at first there was no change in the color of the reagent, nor could crystals of cuprous oxide be seen in these yellowish-green globules with the microscope; a precipi-

tate of cuprous oxide began to form after boiling for a minute or so; and upon further boiling a copious precipitate formed. Other sections mounted in Fehling's solution on hollow-ground slides were placed in the electric oven and examined daily. After twenty-four hours cuprous oxide crystals had formed, and for three days thereafter the number of crystals gradually increased and the globules disappeared. The slow reduction of two tests suggest the presence of glucosides in these globules.

The presence of resin was not indicated in sections which had been left in concentrated copper acetate solution for three weeks.

The blue stain characteristic of mucilage did not develop in free-hand sections placed in a solution of methylene blue in equal parts of alcohol, glycerine and water.

Tannin was not demonstrated in sections mounted in ferric chloride.

A few crystals of cuprous oxide were formed in the cells of the epidermis, and also in cells of the collenchyma, parenchyma, and starch sheath of the primary cortex, in sections mounted in Fehling's solution and heated just to the boiling point. Crystals of cuprous oxide were also formed in the phloëm cells of the primary vascular bundles. The presence of reducing sugar, probably grape sugar, was thus indicated by the immediate reduction of the reagent without boiling.

Starch was not generally found in the starch sheath; a slight amount of starch was found in region II; a few cells of the starch sheath in the main axis of the stem contained much starch.

The contents of cells of the phloëm of the primary vascular bundles and the phloëm of the anomalous tissue stain red in sections left in Millon's reagent for several hours in the electric oven. A yellow stain of these cell contents appeared in sections mounted in concentrated nitric acid, and the yellow color deepened when a drop of ammonium was added. The results of the two tests indicate the presence of proteins.

Large spherical aggregates of calcium oxalate crystals are present in the starch sheath cells, in the outer rows of cells of the pericycle, and in the pith cells, in all regions of stem; but are especially abundant in region III and below (fig. 9, *s*). The starch sheath cells and a few collenchyma cells of the primary cortex in region II are filled with masses of the crystals. Only one aggregate of crystals occurs in a single cell. A few of the pith cells in regions III and IV and the main axis contain a few or quite a number of single crystals (fig. 9, *t*). There are abundant clusters of these

crystals in the secondary medullary rays and pericycle cells in the zones of thin-walled anomalous tissue (designated as phloëm zones in this article). When sections mounted in distilled water on a slide were irrigated with a drop of hydrochloric acid, the crystals disappeared in several seconds without effervescence, indicating the presence of calcium oxalate. Jost ('08) states, many plants normally containing calcium oxalate have only a small amount or none present, when presented with not more than the indispensable minimum of $Ca(NO_3)_2$, according to Amar; or if presented with nitric acid in the form of an ammonium salt, according to Beneck. It is of interest to note here that Politis (*fide* Experiment Station Record, '12) draws the following conclusions from his research work on the origin and office of oxalate of lime in plants: "Oxalic acid, with its resulting calcium oxalate, has its origin in the cell in which the salt is found in crystalline form, and the acid is formed by oxidation of glycogen or amyloids."

Free-hand sections over one cell in thickness were tested for alkaloids, and sections soaked in an alkaloid solvent (one part of tartaric acid dissolved in twenty parts of alcohol for twenty-four hours and rinsed in distilled water for twenty-four hours were used as a control. No precipitate formed in sections mounted in potassium iodide-iodine, potassium permanganate, or ammonium molybdate. Sections were treated with picric acid and tannic acid, as suggested by Winterstein and Trier ('10), but no precipitate formed. No characteristic color developed with nitric acid or sulphuric acid to indicate the presence of alkaloids. No precipitations or color reactions occurred in the control sections treated with tartaric alcohol, nor was there any marked indication that cell contents had been dissolved out.

The Root.

Tests were made on the sections of the root as on the sections of the stem. Globules in the cells of the epidermis and primary cortex dissolved out in xylene or chloroform, and the globules of other sections stained red in Sudan III and pink in alcannin. The globules did not give a test for saponifying oil nor resin. A slight amount of reducing sugar was found in the cells of the pericycle, the phloëm and xylem parenchyma of the primary vascular bundles, and the secondary medullary rays and phloëm of the anomalous tissue. The presence of protein was indicated in the cell contents of the phloëm of the primary vascular bundles and the phloëm of the anomalous tissue by the red stain with Millon's

reagent. The characteristic yellow stain deepened when a drop of ammonium was added to sections which had been mounted in nitric acid. Clusters of crystals of calcium oxalate were numerous in the cells of the primary cortex, the pericycle, and the secondary medullary rays. No volatile oil, glucosides, mucilage, tannin, starch nor alkaloids were found to be present in the root.

The Leaf.

Similar tests were made on sections of the leaf. Certain globules which stain red in Sudan III and pink in alcannin, like oil, were present in the cells of the epidermis, and photosynthetic tissue (fig. 73, *q'*). These globules stain unevenly like similar globules in the stem. The globules are soluble in xylene, chloroform, and ether. Other tests did not indicate the presence of saponifying oil nor resin in these globules.

There are other globules, most of which are quite large, in the epidermal cells, which did not stain with Sudan III nor alcannin, and which were not soluble in xylene, chloroform, nor ether; but these globules in sections left in the electric oven formed crystals of cuprous oxide with Fehling's solution continuously for three days, the presence of glucosides thus being indicated (fig. 73, *r'*).

The presence of reducing sugar was demonstrated by the formation of cuprous oxide crystals in the cells of the phloëm and photosynthetic tissue when sections of the leaf in Fehling's solution were heated to the boiling point.

The characteristic stain of protein developed with Millon's reagent, or nitric acid with the addition of ammonium, in the cell contents of the phloëm of leaf sections tested.

Clusters of calcium oxalate crystals occur in great abundance in the border parenchyma cells of the veins, and the water-storage tissue. In a general drawing of a bleached leaf, the greater part of the leaf is darkened by the numerous crystals mapped out along the veins and veinlets of the leaf (fig. 96).

The other tests applied did not indicate the presence of volatile oil, mucilage, tannin, starch nor alkaloids in the leaf.

The Hairs of the Leaf and Stem.

In the cells of the basal portions of the clothing hairs (figs. 16, *q'*, and 77, *c''*,) and of the glandular hairs (figs. 17, *q'*, and 78, *c''*) of the stem and the leaf are globules which stain with Sudan III and alcannin.

There are more of such globules in the four cells of the terminal portion (figs. 17, *u'*, and 78, *u'*) of the glandular hairs than in the basal portion (figs. 17, *u'*, and 78, *u'*). In the terminal cell, and the cell just beneath, in certain of the glandular hairs are a few very large oil globules (fig. 78, *d''*) some of which have a volume thirty-six times as great as the other oil globules. The usual tests do not indicate the presence of saponifying oil, volatile oil nor resin in these globules. The cell contents of the terminal portion of the glandular hairs appear yellow in formalin material. In iodine solution, potassium iodide-iodine or chloroiodide of zinc a portion of these cell contents stain dark blue. The stain with iodine solution thus indicates the presence of starch.

The cell contents of the terminal portion of the glandular hairs did not dissolve out in 5 per cent potassium hydroxide in twenty-four hours or one week. After remaining twenty-four hours in concentrated potassium hydroxide solution all the oil globules and most of the cell contents had been dissolved out. Portions of the cell contents dissolved out in 10 per cent nitric acid and 10 per cent sulphuric acid. In concentrated sulphuric acid the cell contents are stained orange, and a part of the cell contents are dissolved out after 15 minutes. A part of the cell contents are dissolved out in 15 minutes in sections treated with concentrated nitric acid, but the cell contents do not change in color in this strength of the acid. Apparently only the oil globules are removed from hairs remaining in xylene, chloroform or ether for twenty-four hours or one week. The cell contents stain with safranin, and portions also stain with hæmatoxylin.

Some reducing sugar was present in the cells of the basal portions of both the clothing and glandular hairs.

The presence of glucosides, mucilage, tannin, protein, calcium oxalate or alkaloids was not demonstrated in either of the two types of hairs.

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DESCRIPTION OF PLATES.

PLATE I.

Fig. 1. A photograph of a main branchlet, showing the small leaves and the habit of branching.

PLATE II.

Fig. 2. Cross section of the stem in region I. (See fig. 29, *m''*); *a*, epidermis; *b*, collenchyma of primary cortex; *c*, parenchyma of primary cortex; *d*, starch sheath between the dotted and continuous line; *e*, outer area of the pericycle made up of small cells; *f*, inner area of the pericycle made up of large cells; *g*, primary medullary ray; *h*, pith; *i*, hair; *j*, primary vascular bundle. × 48.

Fig. 3. Cross section of the stem in region II. (See fig. 29, *o''*.) The woody cylinder is colored black, and the included phloëm groups and larger tracheæ are left white. The cell walls are cellulose in the area *e''*, which is the outer portion of the pericycle area *e*, and are lignified in the inner portion *e''*, of the pericycle area *e*. Corresponding parts lettered as in fig. 2: *k*, leaf-trace bundle in the pericycle. × 48.

Fig. 4. Cross section of the stem in region III. (See fig. 29, *p''*). The woody cylinder is colored black, and the included phloëm groups and larger tracheæ are left white. Corresponding parts lettered as in fig. 2: *b*, leaf-trace bundle; *l*, inner border of the innermost anomalous xylem ring; *m*, anomalous xylem; *n*, active arc of secondary meristem. × 30.

Fig. 5. Cross section of the stem in region IV. (See fig. 29, *r''*.) The cell walls of the primary medullary rays, and regions *e''* and *f*, of the pericycle in this section, are not lignified except in the outer portion of the pericycle area *e''* (this outer portion of *e''*, colored black, being included in the

woody cylinder). Corresponding parts lettered as in figs. 2 and 4: *n*, one of the several active arcs of secondary meristem in the figure which anastomose radially and tangentially with the arcs, in the corresponding ring of secondary arcs, in the region of stem below; *o*, area of one to six rows of very large parenchyma cells of the primary cortex around the stem; *p*, anomalous zylem zone; *q*, zone of anomalous tissue, designated as a phloëm zone in this article; *r*, secondary medullary ray; *s*, anomalous phloëm; *t*, pericycle cells in outer portion of a zone of anomalous tissue, designated as a phloëm zone in this article. × 18.

PLATE III.

FIG. 6. Cross section of the stem in region V. (See fig. 29, *s''*.) Corresponding parts lettered as in fig. 5: *u*, radial strip of xylem anastomosing from one anomalous xylem zone to another; *a*, lenticel; *b'*, cork; *c'*, xylem, the cell walls of which are not lignified except those of the tracheæ included in the innermost anomalous xylem zone; *d'*, xylem of usual structure included in the innermost xylem zone; *f'*, bast fiber; *g'*, radial strip of tissue anastomosing from one phloëm zone to another. × 9.

FIG. 7. Cross section of the stem in region I. (See fig. 29, *m''*.) Corresponding parts lettered as in fig. 2: *h'*, phloëm of primary vascular bundle; *i'*, procambium of primary vascular bundle; *j'*, primary xylem of primary vascular bundle; *k'*, cuticle; *l'*, chloroplast. × 225.

FIG. 8. Cross section of the stem in region II. (See fig. 29, *o''*.) Corresponding parts lettered as in figs. 2 to 7, inclusive: *m'*, secondary phloëm of primary vascular bundle; *n'*, cambial layer of primary vascular bundle; *o'*, secondary xylem of primary vascular bundle; *p'*, intercellular space. × 225.

PLATE IV.

FIG. 9. Cross section of the stem in region III. (See fig. 29, *p''*.) Corresponding parts lettered as in fig. 8: *k*, parenchyma of primary cortex between collenchyma of the rib and the starch sheath; *q'*, oil globule; *r'*, glucoside globule; *s'*, mass of calcium oxalate crystals in a pith cell; *t'*, single crystals of calcium oxalate in a pith cell. × 225.

FIG. 10. Outer portion of cross section of the stem in region IV. (See fig. 29, *r''*.) Corresponding parts lettered as in fig. 8: *o*, area of one to six rows of very large parenchyma cells of the primary cortex around the stem; *p*, anomalous xylem zone; *q*, anomalous phloëm zone; *r*, secondary medullary ray; *s*, anomalous phloëm; *t*, pericycle cells in the outer area of a zone of anomalous tissue, designated as a phloëm zone in this article; *u*, radial strip of xylem anastomosing from one anomalous xylem zone to another. × 225.

PLATE V.

FIG. 11. Inner portion of a cross section of the stem in region IV. (See fig. 29, *r''*.) Corresponding parts lettered as in fig. 8. × 225.

FIG. 12. Surface view of lengthened epidermal cells of a rib of the stem in region III, showing type and frequency of stomata. × 225.

FIG. 13. Radial longitudinal section of the epidermis of the stem in region III: *k'*, cuticle. × 225.

FIG. 14. Surface view of the epidermal cells between the ribs of the stem in region III, showing type and frequency of stomata. × 225.

FIG. 15. Portion of radial longitudinal section of the stem in region III, showing epidermis, *a*, the parenchyma of the primary cortex, *e*, and the starch sheath, *d*: *k'*, cuticle; *p'*, intercellular space. × 225.

FIG. 16. Clothing hair of the epidermis of the stem in region I: *a*, basal cell; *k'*, cuticle; *q'*, oil globule; *u'*, terminal portion; *a''*, basal portion of one or more short cells above the basal cell. × 330.

FIG. 17. Glandular hair of the epidermis of the stem in region I: *a*, basal cell; *k'* cuticle; *u'*, terminal portion; *a''*, basal portion consisting of two or more short cells; *b''*, dense cell contents in terminal portion; *c''*, small oil globule; *d''*, large oil globule. × 225.

FIG. 18. Longitudinal section of the collenchyma of the primary cortex of the stem in region III. × 225.

FIG. 19. Portion of cross section of the stem in region V, showing cork cells, *b'*: *d*, starch sheath; *o*, area of one to six rows of very large parenchyma cells of the primary cortex around the stem; *q'*, bast fiber. × 225.

FIG. 20. Portion of cross section of the stem in region V, showing lenticel, *b'*: *a*, epidermis; *c*, parenchyma of the primary cortex; *d*, starch sheath; *o*, area of one of six rows of very large parenchyma cells of the primary cortex around the stem; *f'*, bast fiber; *k'*, cuticle; *p'*, intercellular space; *f''*, stoma. × 225.

PLATE VI.

FIG. 21. Portion of longitudinal section of the stem in region III: *d*, starch sheath; *e'*, outer portion of the pericycle, the cell walls of which have not lignified, corresponding to the area of pericycle *e'* in cross section, fig. 9. × 225.

FIG. 22. Portion of cross section of the stem in region V to show veriation in location of bast fibers in the pericycle: *d*, starch sheath; *o*, portion of area of one to six rows of very large parenchyma cells of the primary cortex around the stem; *f'*, bast fibers; *e'*, outer area of the pericycle, the cell walls of this area not having lignified. × 225.

FIG. 23. Longitudinal section of bast fiber of medium length, from the outer portion of the pericycle of the stem in region IV, corresponding in location to the area of pericycle *e'* in cross section, fig. 9. × 225.

FIG. 24. Macerated wood fibers of medium size from the anomalous xylem of the stem in region IV. × 225.

FIG. 25. Longitudinal section of cells in the central portion of the pericycle from region III of the stem, corresponding to the area of pericycle, *e''*, fig. 10, in region IV. × 225.

FIG. 26. Portion of cross section of the stem in region V, showing the areas of pericycle *e''* and *f*, and cells of the inner portion of the anomalous xylem zone, *c'*, the cells of which in this region of stem resemble pith in shape in cross section: *q*, tissue designated as an anomalous phloëm zone in this article; *r*, secondary medullary ray; *s*, anomalous phloëm; *t*, pericycle cells in the outer part of the tissue designated as an anomalous phloëm zone in this article; *c'*, anomalous xylem having cellulose walls and located in the

innermost anomalous xylem zone; *d'*, anomalous xylem of the usual structure in the outer portion of the innermost anomalous xylem zone. × 225.

FIG. 27. Longitudinal section of primary medullary ray cells from region III of the stem. × 225.

FIG. 28. Longitudinal section of pith cells from region III of the stem. × 225.

PLATE VII.

FIG. 29. Diagram of a longitudinal section of the main axis of the stem to show the location of the five regions of stem, the broken leaf traces of region V and below, and the anomalous xylem-phloëm zones; *j*, primary vascular bundle; *n*, arcs of secondary meristem anastomosing radially with the arcs, in the corresponding ring of secondary arcs, in the region of stem below; *p*, anomalous xylem zone; *q*, tissue designated in this article as an anomalous phloëm zone; *u*, radial strip of xylem anastomosing from one anomalous xylem zone to another; *g'*, radial strip of tissue anastomosing from one anomalous phloëm zone to another; *h''*, fruit; *i''*, leaf; *j''*, branch; *k''*, region of primordial meristem; *l''*, procambium; *m''*, region I; *n''*, region of the stem for fig. 31; *o''*, region I; *p''*, region III; *q''*, region of the stem for figs. 50 and 51; *r''*, region IV; *s''*, region V; *t''*, leaf trace of leaf retained; *u''*, broken leaf trace.

FIG. 30. Primary vascular bundle in the formation in region I of the stem: *f*, inner area of pericycle; *g*, primary medullary ray; *h*, pith; *h'*, primary phloëm; *i'*, procambium; *j'*, primary xylem; *a'''*, water tube.

FIG. 31. Cross section of the stem just below region I (location shown in fig. 29, *n''*), showing young leaf trace bundle, *k*, in pericycle. Corresponding parts lettered as in fig. 8. × 225.

FIG. 32. Primary vascular bundle with cambial layer from region II of the stem. Corresponding parts lettered as in fig. 8. × 330.

FIG. 33. Longitudinal section of sieve tubes from secondary phloëm of primary vascular bundle in region III of the stem. × 330.

FIG. 34. Longitudinal section of companion cells, *b'''*, and phloëm parenchyma *c'''*, of a primary vascular bundle in region III of the stem. × 330.

FIG. 35. Primary vascular bundle, in which the cambial layer is no longer active, in region III of the stem. Corresponding parts lettered as in fig. 32: *d'''*, sieve tube having thickened cell walls; *f'''*, sieve tube with cell walls not thickened. × 330.

FIG. 36. Longitudinal section of a small spiral tracheal tube from primary xylem in region III of the stem. × 225.

FIG. 37. Longitudinal section of larger spiral tracheal tube from primary xylem in region III of the stem. × 225.

FIG. 38. Longitudinal section of annular tracheal tube of medium size from primary xylem in region III of the stem. × 225.

FIG. 39. Longitudinal section of pitted tracheal tube of medium size from a primary vascular bundle in region III of the stem. × 225.

FIG. 40. Longitudinal section of reticulate tracheal tube of medium size from a primary vascular bundle in region III of the stem. × 225.

FIG. 41. Longitudinal section of wood parenchyma from secondary xylem of primary vascular bundle in region III of the stem. × 225.

FIG. 42. Longitudinal section of pitted fiber tracheids from the secondary xylem of a primary vascular bundle in region III of the stem. × 225.

PLATE VIII.

FIG. 43. Primary vascular bundle from region IV of the stem. Corresponding parts lettered as in fig. 32. × 330.

FIG. 44. Older leaf-trace bundle in the pericycle of the stem in region IV. Corresponding parts lettered as in fig. 32. × 330.

FIG. 45. Portion of cross section of the stem in region V, showing primary medullary ray cells which, in this region of stem and below, resemble pith cells in shape and nature of cell walls: *j*, portion of primary vascular bundle. × 225.

FIG. 46. Longitudinal section of cells in a portion of the pericycle of the stem in region III corresponding in location to the area of pericycle *e''*, fig. 9. × 225.

FIG. 47. Portion of a cross section of the stem in region III: *d*, starch sheath; *e'*, outer area of pericycle; *e''*, central area of the pericycle; *n*, active arc of secondary meristem; *f'*, bast fiber. × 330.

FIG. 48. Portion of a cross section of the stem in region III; *d*, starch sheath; *e'*, outer area of the pericycle; *e''*, central area of the pericycle; *k*, leaf-trace bundle in the pericycle; *n*, active arc of secondary meristem. × 330.

FIG. 49. Portion of a cross section of the stem in region III: *d*, starch sheath; *e'*, outer area of the pericycle; *e''*, central area of the pericycle; *f*, inner area of the pericycle; *m*, anomalous xylem; *n*, active arc of secondary meristem; *s*, anomalous phloëm. × 330.

PLATE IX.

FIG. 50. Cross section of the stem between regions III and IV (location shown in fig. 29, *q''*). Corresponding parts lettered as in fig. 6: *l*, inner border of innermost anomalous xylem zone. × 48.

FIG. 51. Cross section of the stem between regions III and IV (location shown in fig. 29, *q''*). Corresponding parts lettered as in fig. 2: *j*, primary vascular bundle; *o*, portion of area of one to six rows of very large parenchyma cells of the primary cortex around the stem; *p*, anomalous xylem zone; *q*, anomalous phloëm zone; *r*, secondary medullary ray cell; *s*, anomalous phloëm; *t*, pericycle cells in outer part of area of tissue designated in this article as an anomalous phloëm zone; *u*, radial strip of anomalous xylem located similar to the strip of xylem anastomosing from one anomalous xylem zone to another in fig. 10, *u*; *p'*, intercellular space; *n''*, area of anomalous tissue, including a radial strip of anomalous xylem and an anomalous phloëm group just exterior; *o''*, long radial strip of anomalous xylem; *p''*, area of anomalous tissue, including a radial strip of anomalous xylem and a secondary medullary ray between the anomalous phloëm groups. × 225.

FIG. 52. Portion of cross section of stem in region IV; to show arc of secondary meristem, *n*, becoming active to form the fourth xylem-phloëm

zone; *d*, starch sheath; *s*, anomalous phloëm; *e'*, outer area of the pericycle; *p*, anomalous xylem zone; *f'*, bast fiber. × 330.

FIG. 53. Portion of a cross section of stem in region V, showing varied structure of the innermost anomalous xylem zone; *p*, anomalous xylem zone; *q*, anomalous phloëm zone; *r*, secondary medullary ray; *s*, anomalous phloëm; *t*, pericycle cells in outer part of area of tissue designated in this article as an anomalous phloëm zone; *m''*, tracheal tube; *n''*, wood parenchyma cell bordering a tracheal tube; *o''*, wood fiber; *p''*, tissue of inner 20 per cent of the anomalous xylem zone, composed entirely of wood fibers; *q''*, tissue of outer 80 per cent of the anomalous xylem zone composed of wood parenchyma and tracheæ. × 225.

FIG. 54. Longitudinal section of cells in the portion of the innermost anomalous xylem zone of the stem in region V, which correspond to the cells in the area, *c'*, fig. 25, but the cells of which have lignified walls and resemble pith cells in shape in cross section. × 225.

FIG. 55. Cross section of cells of anomalous xylem (shown in longitudinal section in fig. 54). × 225.

FIG. 56. Portion of cross section of stem in region V, showing strip of anomalous xylem, *u*, including a water tube, *M''*, and anastomosing from one anomalous xylem zone to another; *p*, anomalous xylem zone; *q*, anomalous phloëm zone; *r*, secondary medullary ray; *s*, anomalous phloëm; *t*, pericycle cells in outer part of tissue designated as a phloëm zone in this article. × 225.

FIG. 57. Portion of cross section of stem in region V, showing portion of the innermost anomalous xylem zone having wood fibers rectangular or almost square and arranged regularly in radial rows: *a'''*, water tube. × 225.

PLATE X.

FIG. 58. Portion of a cross section of the stem in region V, showing *g'''*, radial row of cells, having a radial dimension two to four times as great as their tangential dimension, in the anomalous xylem-phloëm zones. The radial row of cells consists of *h'''*, pericycle cells, and *a'''*, secondary medullary ray cells, in the anomalous phloëm zones; and of *j'''*, cells, which resemble wood parenchyma in size and proportion, in the xylem zones: *p*, anomalous xylem zone; *q*, zone of tissue designated in this article as an anomalous phloëm zone; *r*, secondary medullary ray; *s*, anomalous phloëm; *t*, pericycle cells in outer area of zone of anomalous tissue, designated in this article as a phloëm zone; *u*, radial strip of xylem anastomosing from one anomalous xylem zone to another; *g'*, radial strip of tissue anastomosing from one anomalous phloëm zone to another. × 225.

FIG. 59. Longitudinal section of a portion of pericycle tissue, located in the outer part of a zone of tissue, designated as an anomalous phloëm zone in this article, and corresponding in location to the area of tissue, *t*, in cross section, fig. 57. × 225.

FIG. 60. Longitudinal section of anomalous tissue, corresponding in location to the strip of anomalous tissue, in cross section in fig. 58: *g'''*, radial strip of tissue anastomosing from one anomalous phloëm zone to another. × 225.

FIG. 61. Cross section of the root in region I: *c*, primary cortex; *f*, pericycle; *g*, primary medullary ray; *j*, xylem of primary vascular bundle; *b'*, cork; *m'*, phloëm of primary vascular bundle; *m''*, tracheal tube. × 48.

FIG. 62. Cross section of the root in region II. Corresponding parts lettered as in fig. 61: *p*, anomalous xylem zone; *q*, zone of tissue designated in this article as an anomalous phloëm zone; *r*, secondary medullary ray; *s*, anomalous phloëm; *t*, pericycle cells in outer part of zone of tissue designated in this article as a phloëm zone. × 48.

PLATE XI.

FIG. 63. Cross section of the root in region III. Corresponding parts lettered as in fig. 62: *n*, active arc of secondary meristem. × 48.

FIG. 64. Cross section of the root in region IV. Corresponding parts lettered as in fig. 63: *g'''*, radial row of cells, having a radial dimension two to four times their tangential dimension, in the anomalous xylem-phloëm zones, and having cellulose cell walls. × 30.

PLATE XII.

FIG. 65. Cross section of the root in region V. Corresponding parts lettered as in fig. 64. × 9.

FIG. 66. Cross section of the root in region I. Corresponding parts lettered as in fig. 62. × 225.

FIG. 67. Cross section of the root in region II. Corresponding parts lettered as in fig. 62: *g'''*, radial row of cells, having a radial dimension two to four times as great as their tangential dimension, in the anomalous xylem-phloëm zones.

PLATE XIII.

FIG. 68. Cross section of the root in region III. Corresponding parts lettered as in fig. 62. × 225.

FIG. 69. Cross section of the root in region IV. Corresponding parts lettered as in fig. 62: *m''*, inner 20 per cent of anomalous xylem zone, made up entirely of wood fibers; *n''*, outer 80 per cent of the anomalous xylem zone, of the usual structure found in anomalous xylem zones of the stem. × 225.

FIG. 70. Portion of cross section of the root in region III, showing arc of secondary meristem, *n*, in the pericycle, *f*. × 330.

PLATE XIV.

FIG. 71. Cross section of the leaf mapping out the zones of tissue: *a*, epidermis; *b*, collenchyma; *c*, photosynthetic tissue; *d*, water-storage tissue; *e*, border parenchyma; *f*, vascular elements, *m'''*, lobe of the leaf. × 48.

FIG. 72. Portion of cross section of the leaf through the main rib. Corresponding parts lettered as in fig. 71: *k'*, cuticle: *m'*, phloëm; *o'*, xylem; *p'*, intercellular space; *d'''*, sieve tube having thick walls; *e'''*, palisade layer of the photosynthetic tissue; *m'''*, mesophyll parenchyma just beneath the palisade layer; *n'''*, mesophyll parenchyma located between the water-storage tissue and the lower epidermis. × 330.

PLATE XV.

FIG. 73. Portion of cross section of the leaf, showing structure between the bases of two lobes on one side of the leaf. Corresponding parts lettered as in fig. 72: *q'*, oil globule; *r'*, glucoside globule; *s'*, mass of calcium oxalate crystals in a cell of the water-storage tissue; *f''*, stoma. × 330.

FIG. 74. Surface view of cells of the upper epidermis, showing type and frequency of stomata. × 225.

FIG. 75. Surface view of cells of lower epidermis, showing type and frequency of stomata. × 225.

FIG. 76. Surface view of the lengthened cells of the upper epidermis over the main rib, showing type and frequency of stomata. × 225.

FIG. 77. Clothing hairs of the epidermis of a very young leaf: *a*, basal cell; *k'*, cuticle, *q'*, oil globule; *u'*, terminal portion; *a''*, basal portion of one or more short cells above the basal cell. × 330.

FIG. 78. Glandular hairs of the epidermis of a very young leaf: *a*, basal cell; *k'*, cuticle; *l'*, dense cell, contents in the terminal spherical cell; *u'*, terminal portion; *a''*, basal portion; *c''*, small oil globule; *d''*, large oil globule. × 330.

PLATE XVI.

FIG. 79. Portion of a tangential section of a leaf bleached in 5 per cent potassium hydroxide: *d*, water-storage tissue; *e*, spiral tracheal tube of the vascular elements; *n'''*, border parenchyma cells having end walls pitted. × 330.

FIG. 80. Surface view of a bleached leaf, embracing the epidermal, the palisade and the vascular bundle systems. × 330.

FIG. 81. Portion of a tangential section of the leaf, showing cells of the epidermis, *a*, and mesophyll parenchyma, *n'''*, under the water-storage tissue: *k'*, cuticle; *p'*, intercellular space. × 330.

FIG. 82. Portion of a cross section of the leaf to show collenchyma in the tip of a lobe of the leaf: *a*, epidermis; *b*, collenchyma; *k'*, cuticle; *n'''*, mesophyll parenchyma. × 80.

PLATE XVII.

FIG. 83. Bleached leaf, showing venation: *d*, water-storage tissue between the three largest veins. × 17.5.

FIG. 84. Blind ending of the venation in the midst of the mesophyll, showing spiral, *p''*, annular, *q''*, and reticulate, *r''*, tracheids. × 330.

FIG. 85. Groups of spiral, *p''*, annular, *q''*, and reticulate, *r''*, tracheids, which extend out close to the expidermis and serve as the ultimate endings of the venation at the edge of the leaf. × 330.

FIG. 86. Portion of a tangential section of the leaf to show pits in end walls of border parenchyma cells, *o'''*, are not conspicuous in unbleached sections: *j*, vascular elements. × 330.

FIGS. 87-89. A series of cross sections of the petiole of the leaf proceeding upward: *a*, epidermis; *b*, collenchyma; *c*, photosynthetic tissue; *d*, water-storage tissue; *e*, border parenchyma; *p''*, *q''*, *r''*, *s''*, and *t''*, five vascular bundles of the petiole.

FIG. 87. Cross section from the base of the petiole. × 48.

FIG. 88. Cross section midway between the base and the tip of the petiole. × 48.

FIG. 89. Cross section from the tip of the petiole, showing vascular-bundle strands, *o''* and *u''*, which are branches of *p''* and *t''*, respectively. ×48.

FIG. 90. Portion of a cross section midway between the base and the tip of the petiole, showing two vascular bundles, *j*: *d*, water-storage tissue; *e*, border parenchyma; *d'''*, sieve tube having thick walls. × 330.

PLATE XVIII.

FIGS. 91 to 94, inclusive. A series of cross sections of the stem in region IV, from below the petiole attachment to a little above. The anomalous tissue is colored black.

FIG. 91. Section of the stem just below the petiole attachment, showing the five leaf-trace bundles, *p''*, *q''*, *r''*, *s''* and *t''*, about to enter the petiole. Corresponding parts lettered as in fig. 2. × 48.

FIG. 92. *p''*, *q''*, *r''*, *s''* and *t''* are in the leaf-trace gap, and are entering the petiole. × 48.

FIG. 93. *p''*, *q''*, *r''*, *s''* and *t''* have left the leaf-trace gap, *p'''*. × 48.

FIG. 94. *p'''*, leaf-trace gap partially closed. × 48.

PLATE XIX.

FIG. 95. Leaf-trace gap closed in a section of the stem just above the petiole attachment. × 48.

FIG. 96. General drawing of a leaf, bleached in 5 per cent potassium hydroxide, to show the abundance of calcium oxalate crystals occurring in the border parenchyma of the veins, and the water-storage tissue. The areas colored black represent the location of the masses of crystals.

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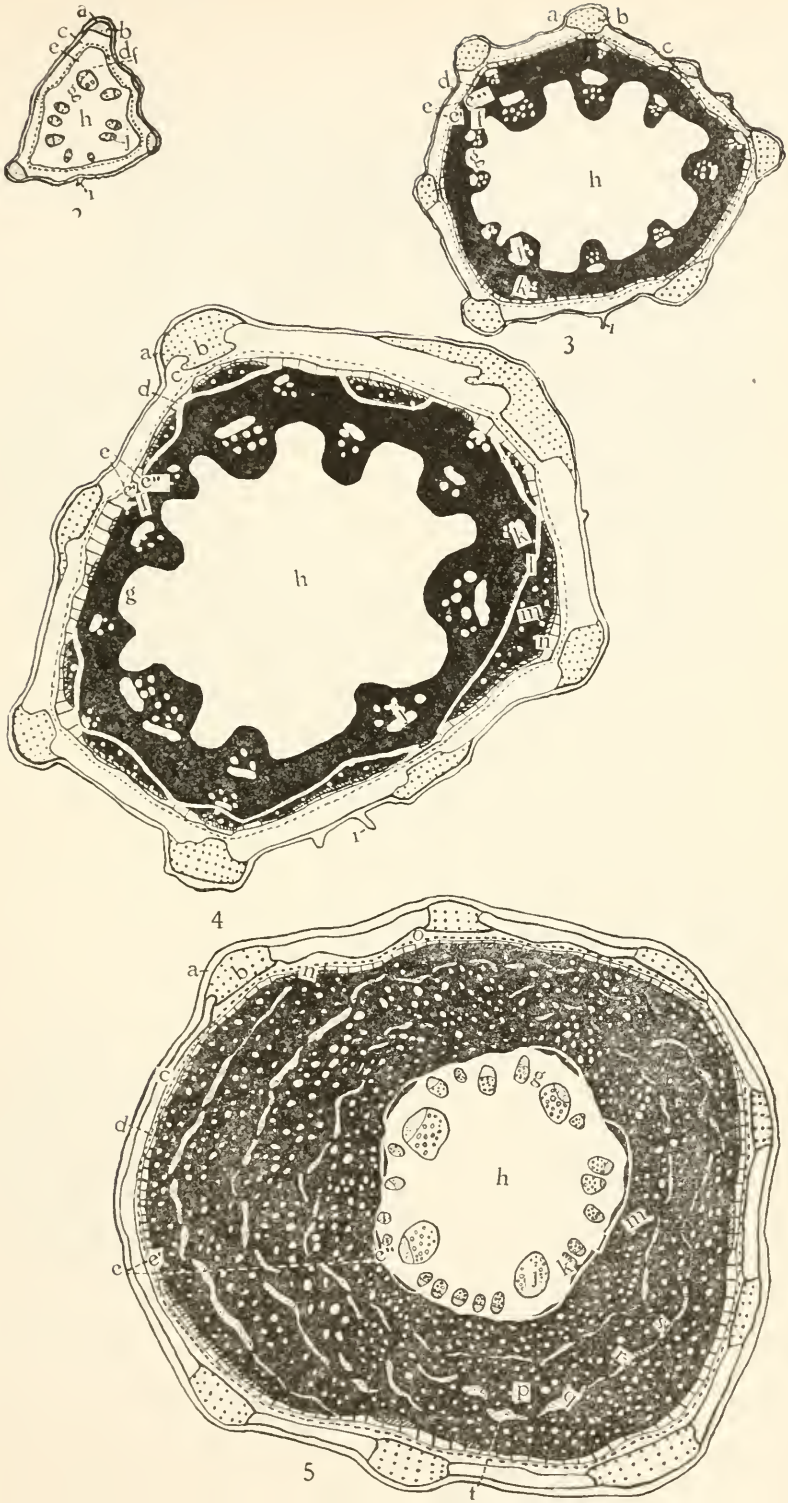
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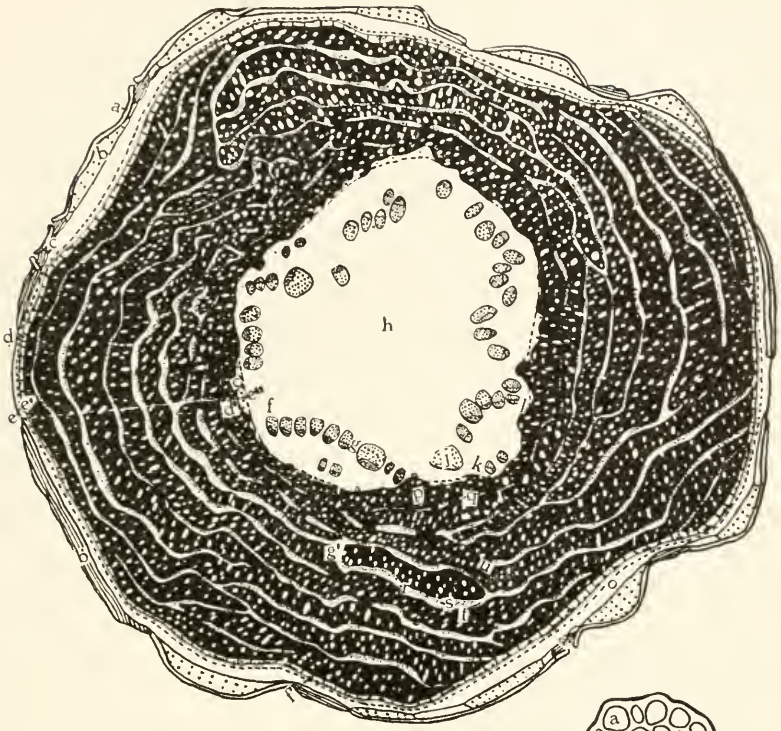

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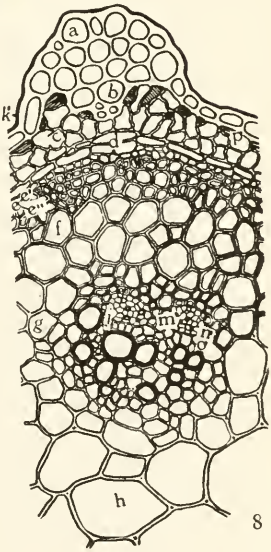

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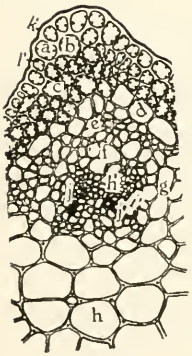

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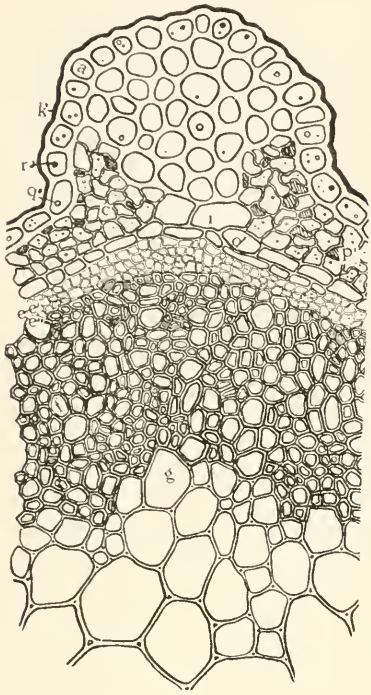

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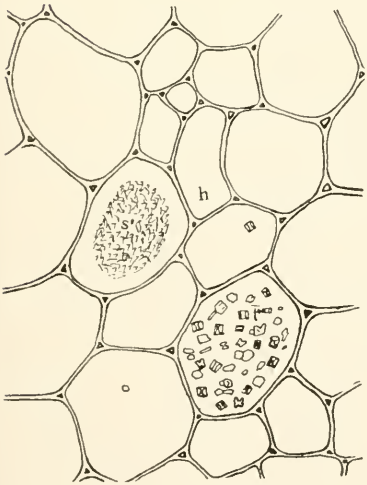

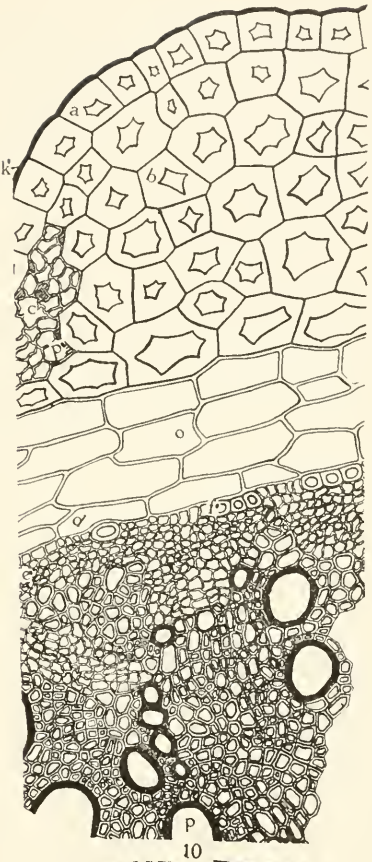

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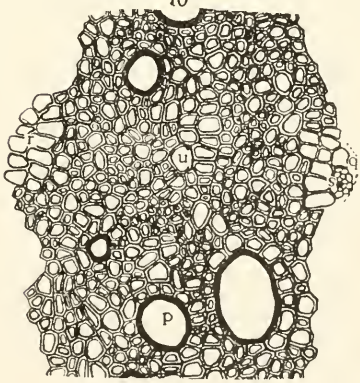

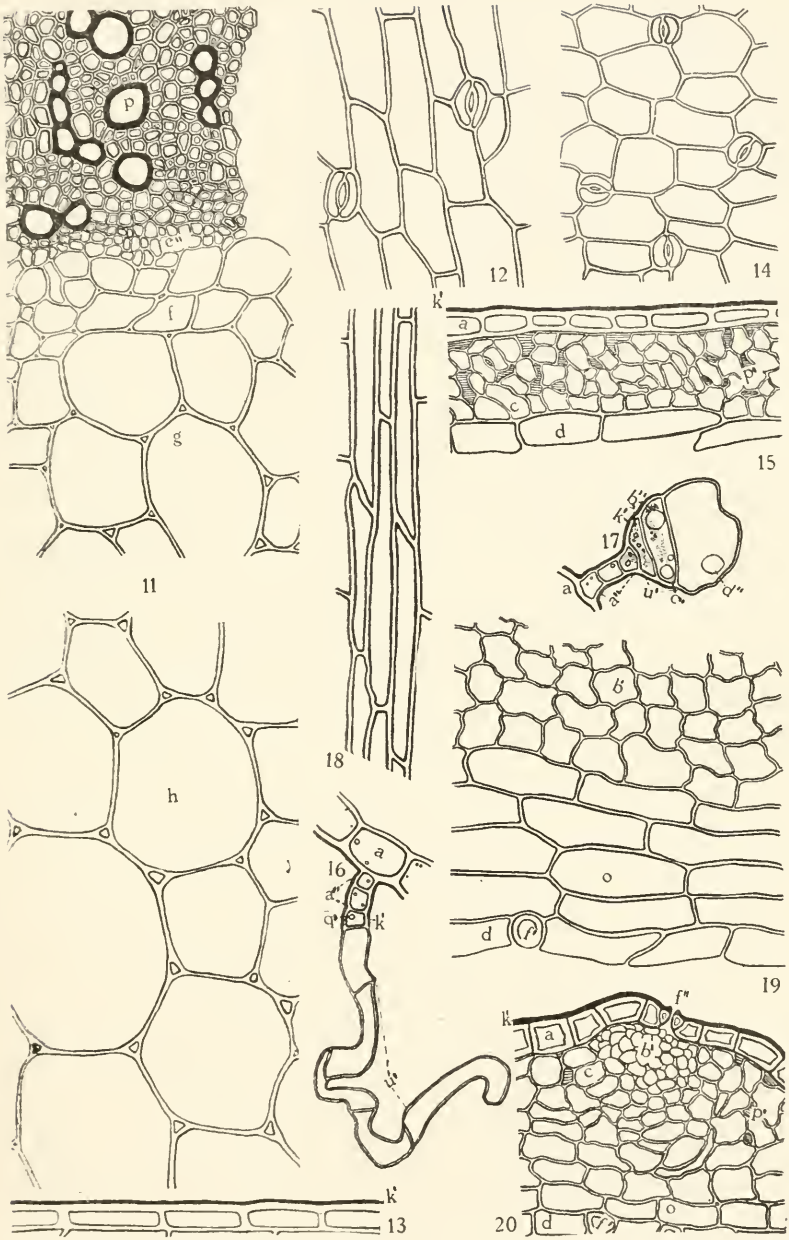
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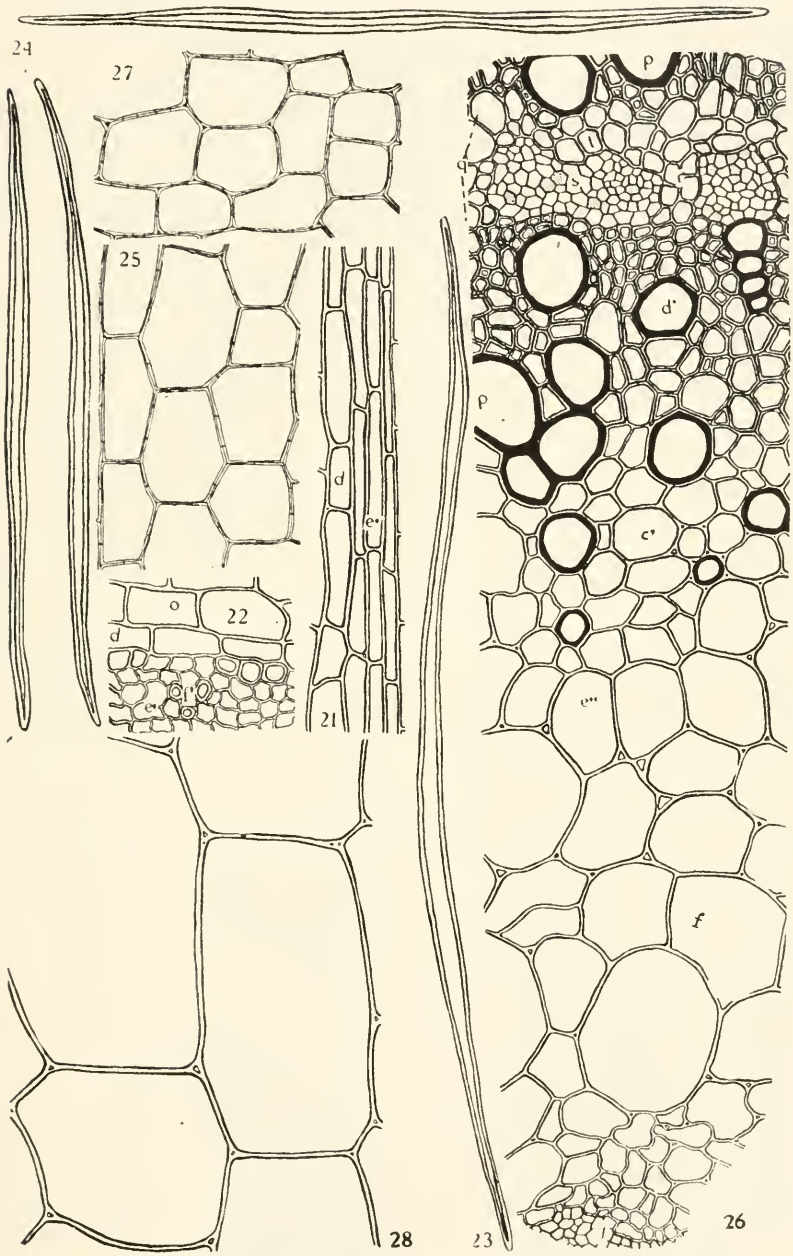
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CYCLOLOMA ATRIPLICIFOLIUM.
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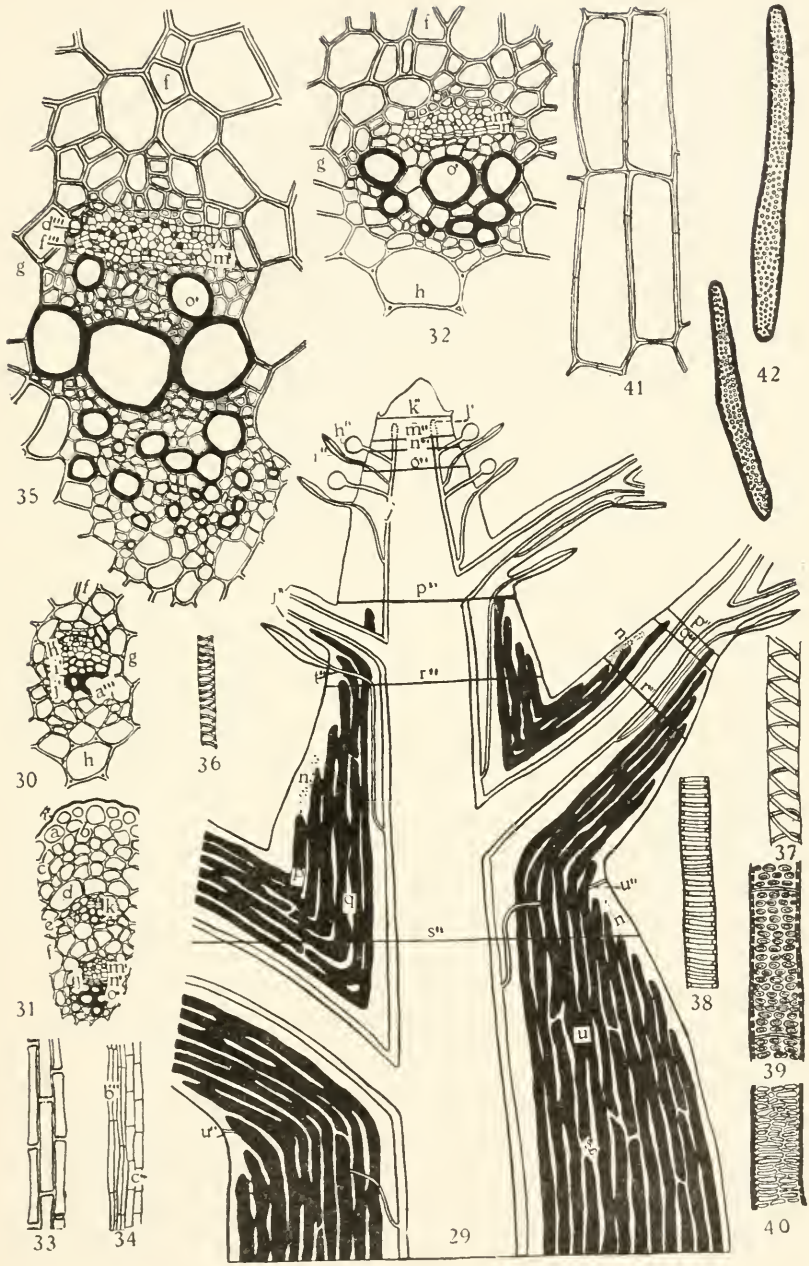
PLATE VII.



Cycloloma Atriplicifolium.
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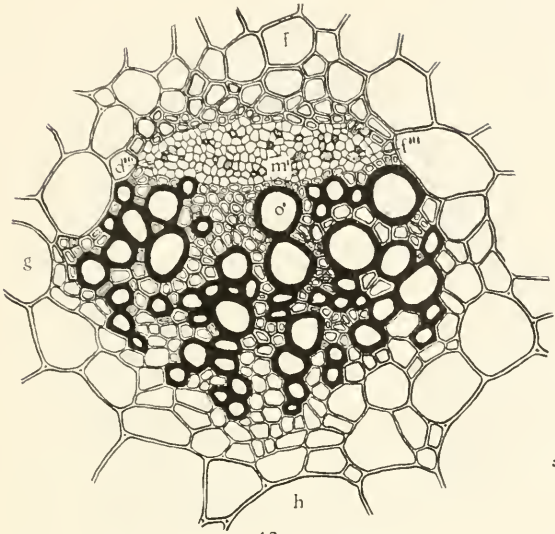
PLATE VIII.



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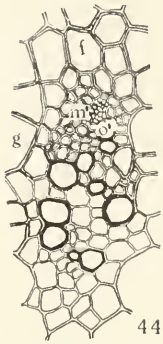

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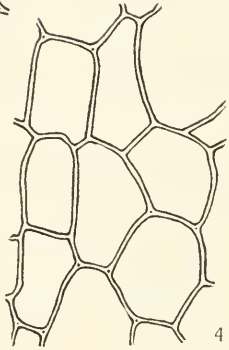

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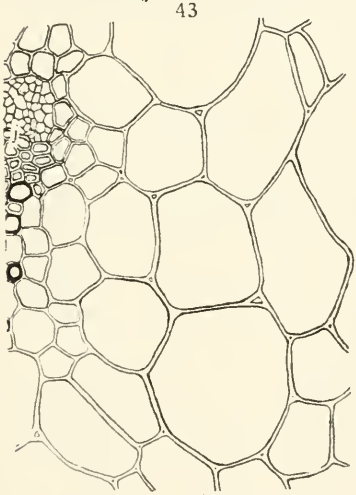

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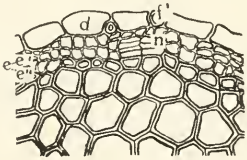

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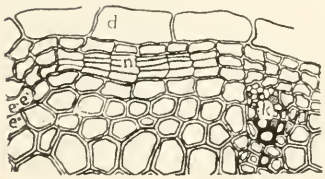

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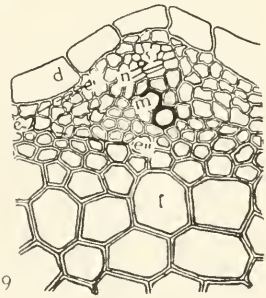

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CYCLOLOMA ATRIPLICIFOLIUM.
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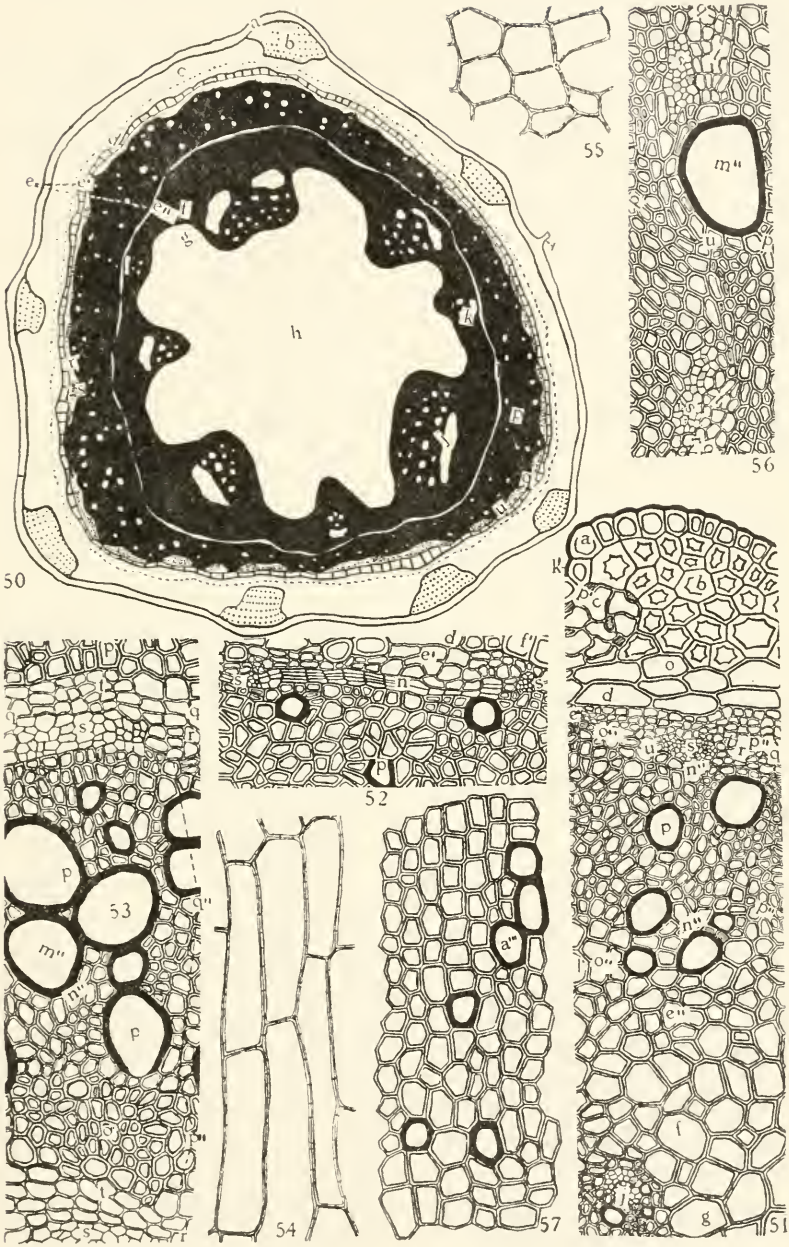
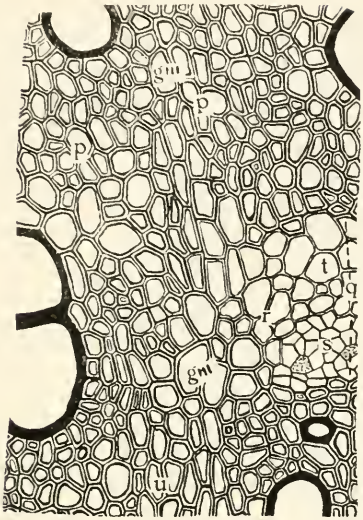
PLATE IX.





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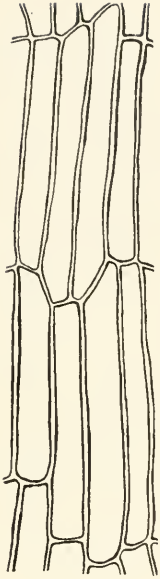

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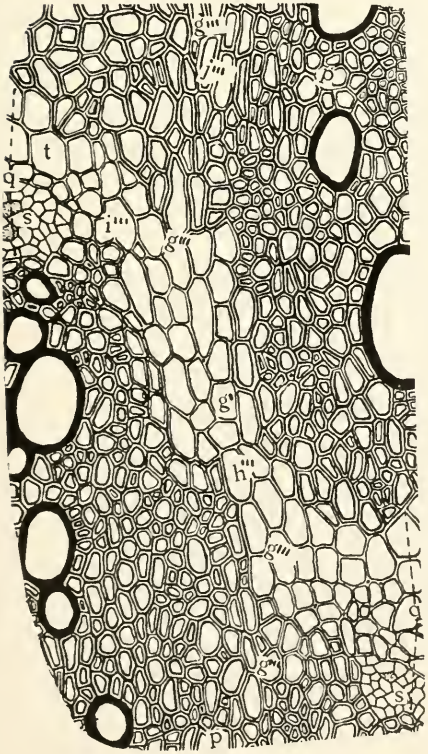

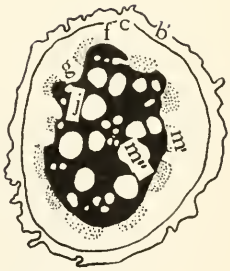

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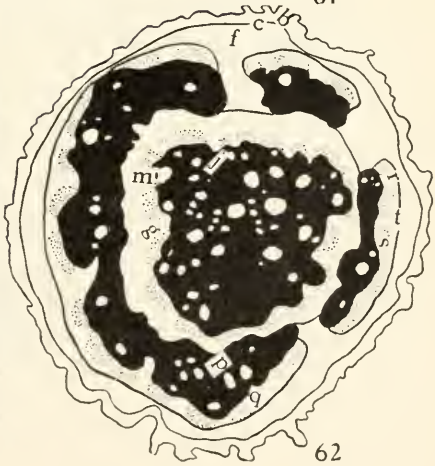

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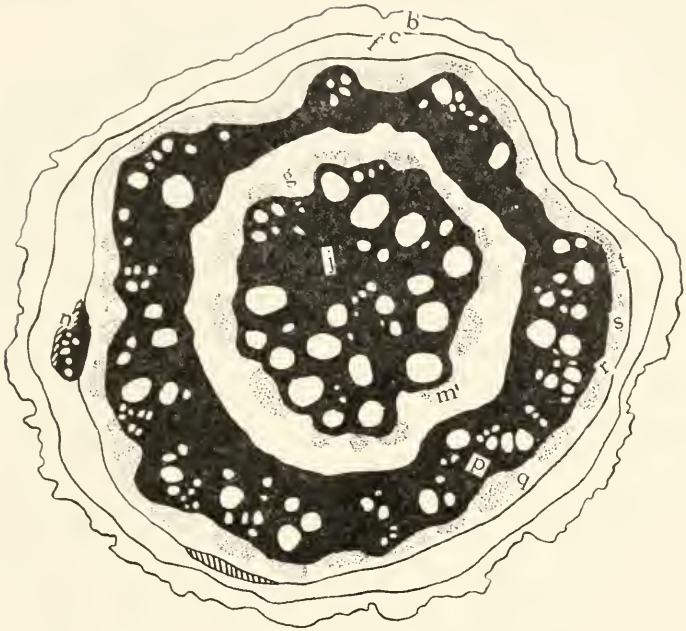

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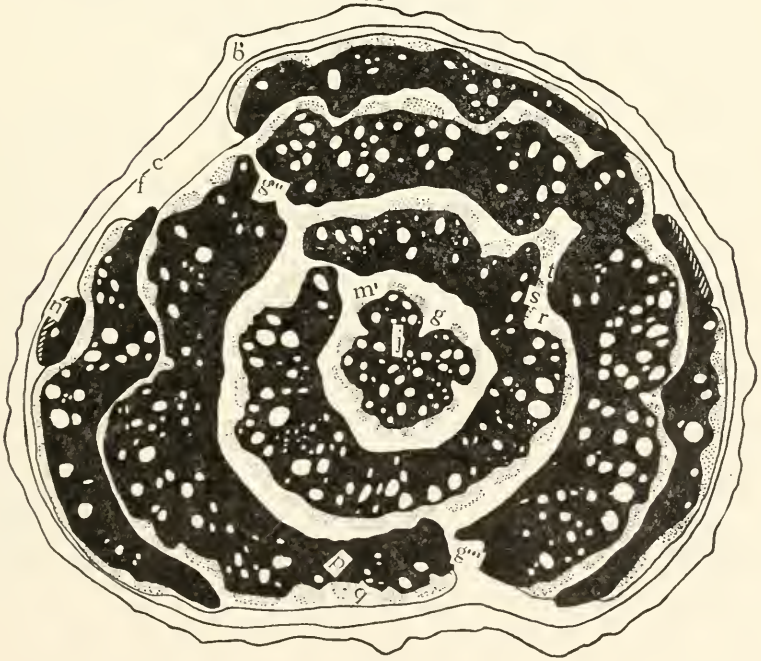

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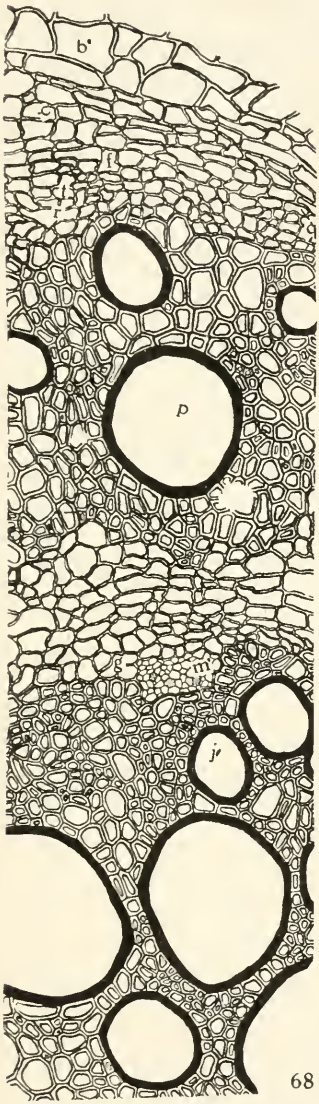

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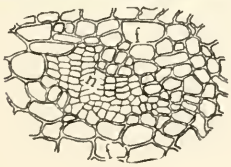

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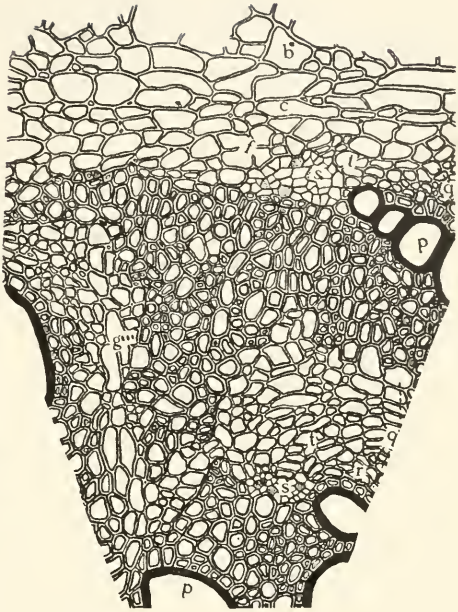

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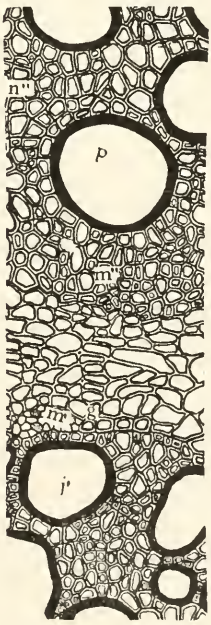

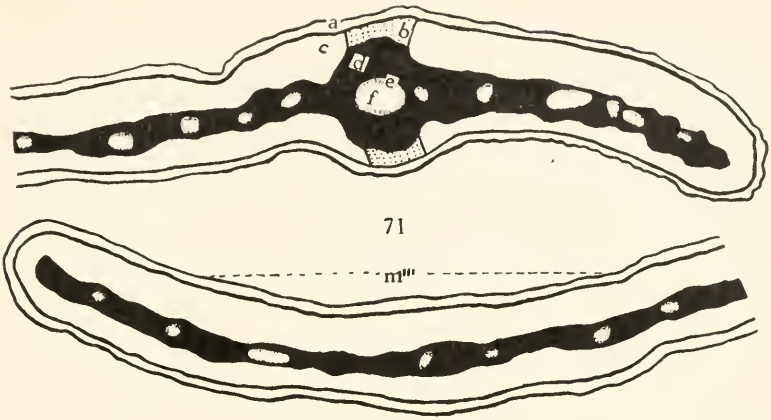

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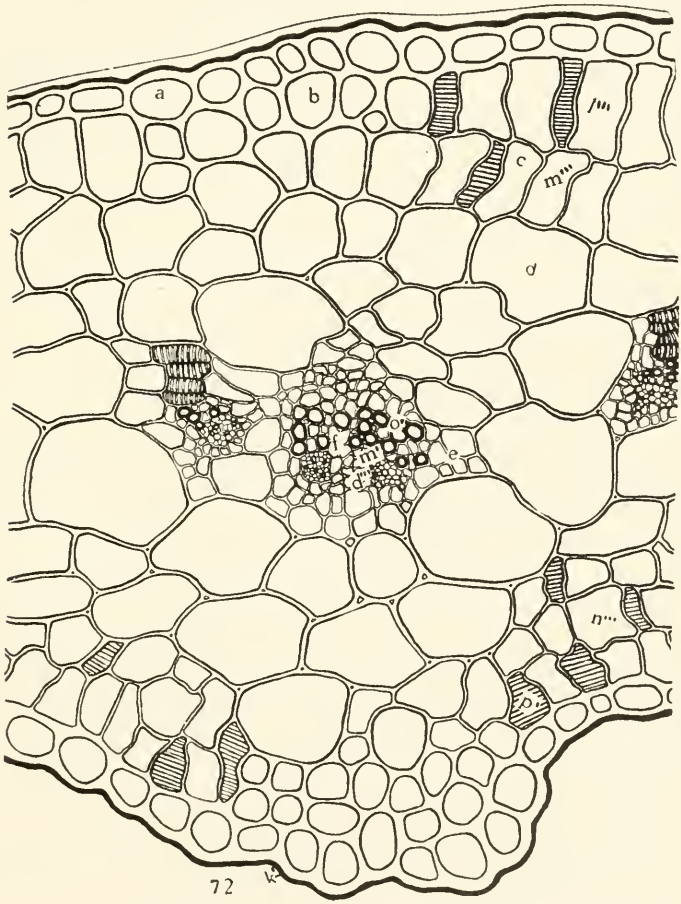

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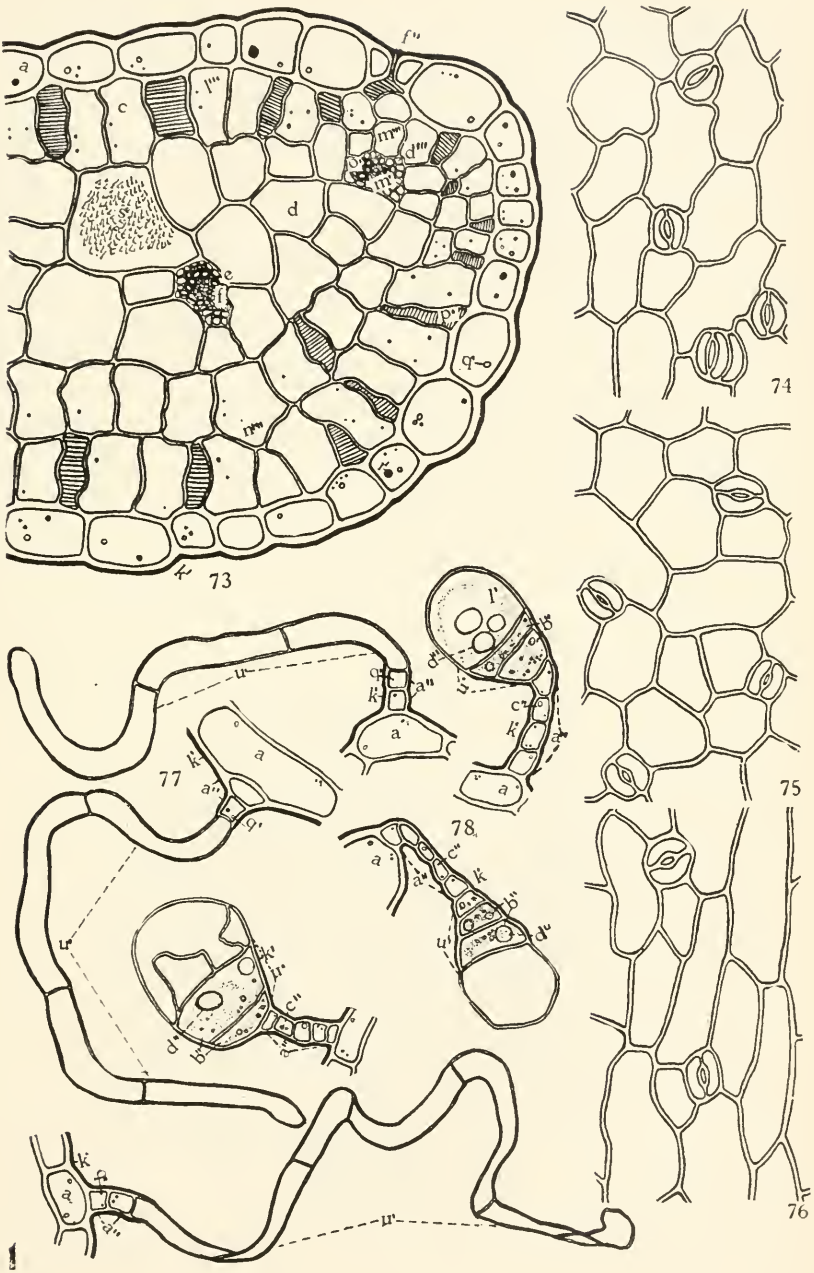
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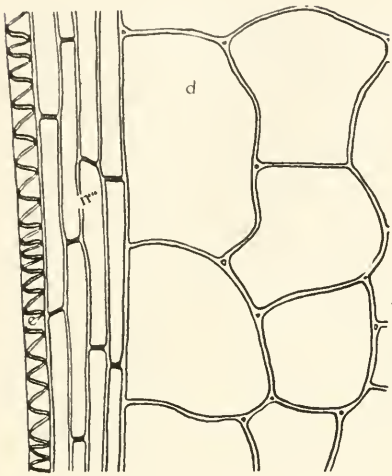
CYCLOLOMA ATRIPLICIFOLIUM.
D. J. Pratt.

PLATE XVI.



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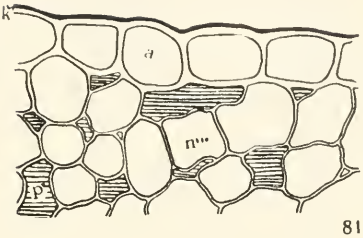

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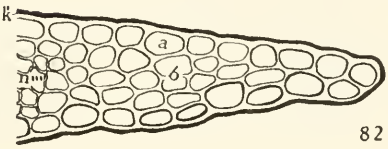

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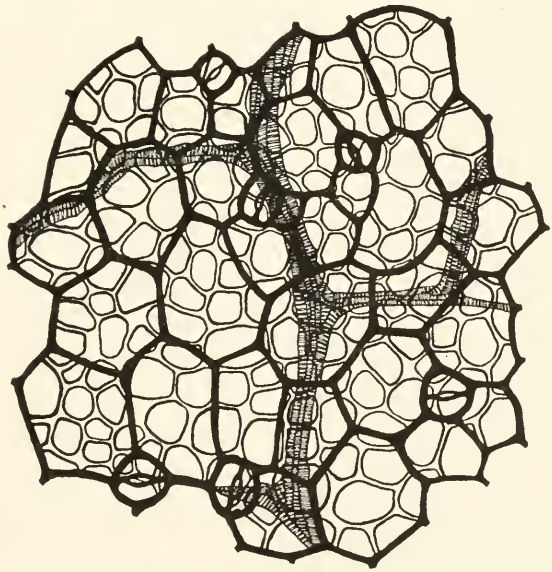

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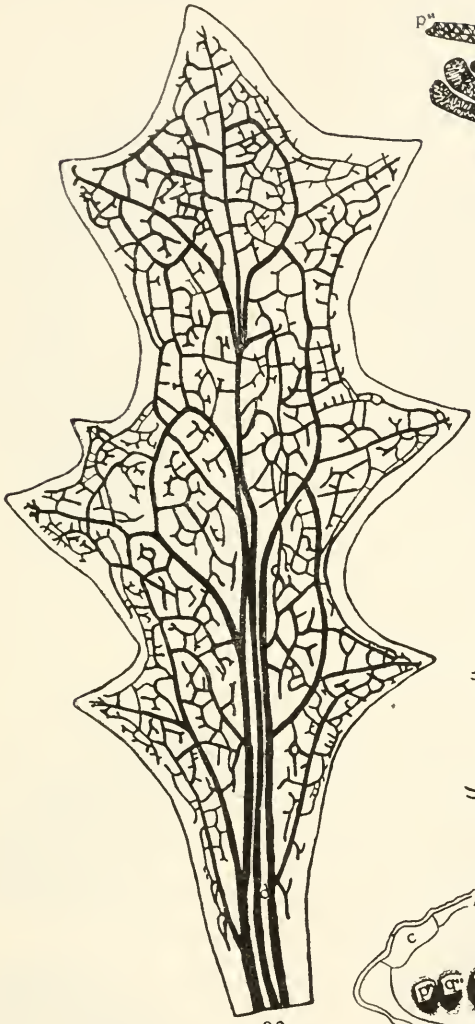

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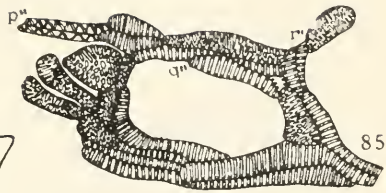

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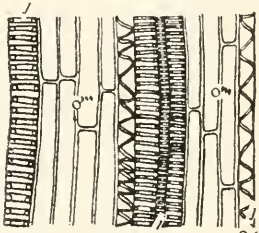

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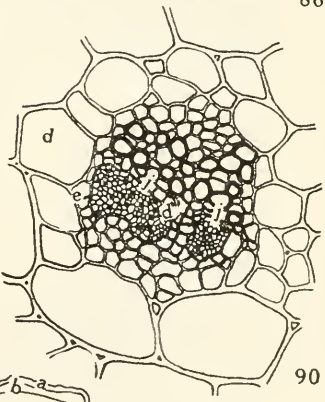

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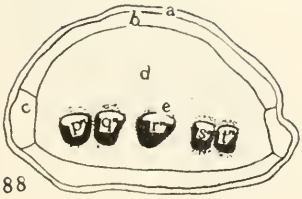

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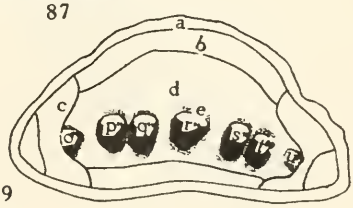

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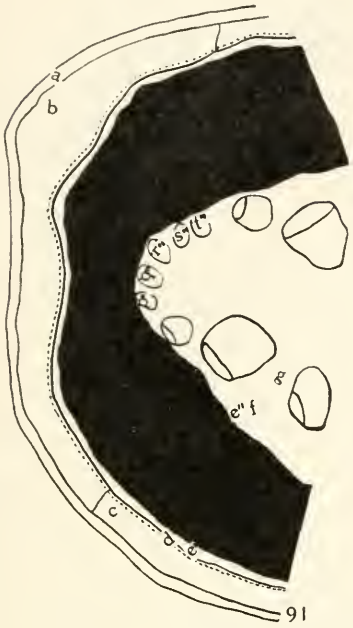
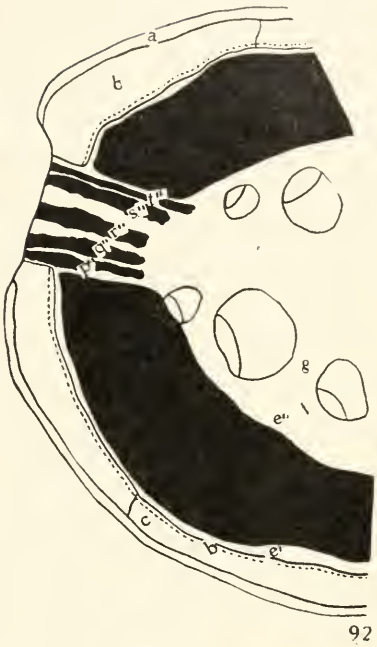
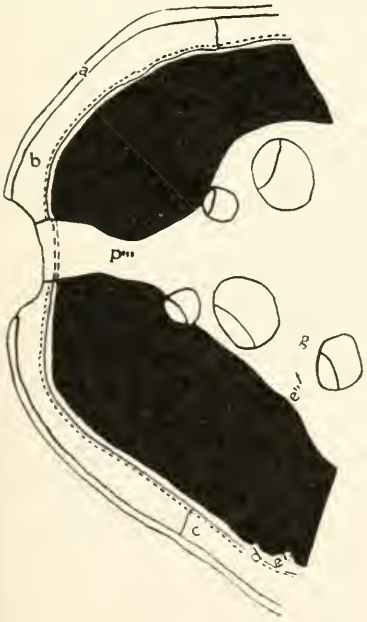
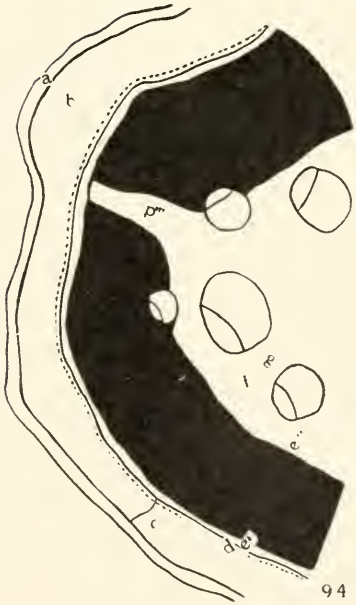
CYCLOLOMA ATRIPLICIFOLIUM.
D. J. Pratt.

PLATE XVIII.



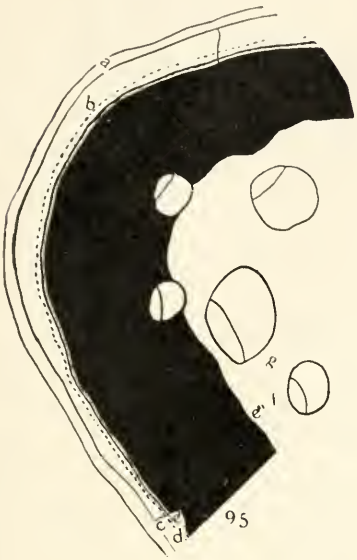


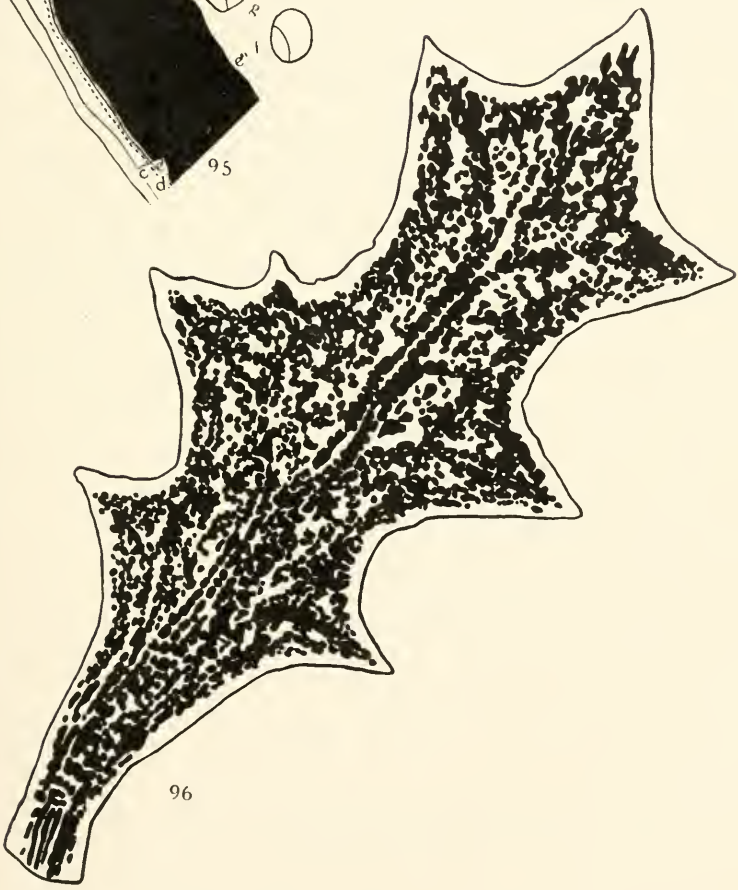




CYCLOLOMA ATRIPLICIFOLIUM.
D. J. Pratt.

PLATE XIX.





THE

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CONTENTS:

LARVAL TREMATODES FROM KANSAS FRESH-WATER SNAILS, *Earl C. O'Roke.*

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THE KANSAS UNIVERSITY SCIENCE BULLETIN.

VOL. X, No. 7.] JANUARY, 1917. [WHOLE SERIES VOL. XX, No. 7.

Larval Trematodes from Kansas Fresh-water Snails.

BY EARL C. O'ROKE.

Thesis submitted in partial fulfillment of the requirements for the degree of master of arts in the Graduate School of the University of Kansas, 1916.

INTRODUCTION.

THIS study of larval trematodes was undertaken by the writer at the suggestion of Prof. Bennet M. Allen, in an attempt to add to the knowledge of this interesting group of parasites.

The snails used in the investigations were collected during the summer and fall of 1915 in connection with the Kansas State Biological Survey, and studies were made of the behavior and habits of the living cercariæ before the specimens were preserved for further morphological studies.

METHODS OF STUDY.

For convenience in transporting live snails, it was found desirable to tie the specimens in cheesecloth bags of twenty-five each, packing loosely in wet excelsior.

In the laboratory the snails were isolated in individual watch glasses and kept covered with water. Normally the cercariæ emerged within two or three days. Some of the infected snails were then preserved entire, and others were crushed for studies of living sporocysts and rediæ.

The fixatives used were a saturated corrosive sublimate solution with one per cent glacial acetic acid, Zenker's fluid, and four per cent formalin. The latter was discarded after trials proved the superiority of the other fixatives. Corrosive sublimate was preferable for free cercariæ.

Several different stains were used. Picrocarmine was excellent for studies of the living cercariæ. Specimens so stained could

sometimes be preserved by drawing glycerine under the cover slip with filter paper. For toto counts, Mayer's hæmalum gave the best results. The sections were cut from five to seven micra in thickness and stained with iron alum hæmatoxylin. Good results were also obtained by staining with hæmalum and using eosin or orange g. for counter stains.

The free cercariæ could be made to expand by heating slightly. When they were in this condition the fixing fluid was poured on suddenly, fixing the specimens in the best form for study.

The measurements given in this paper were taken usually from mounted specimens, and are not as accurate as those from living forms would be, because of shrinkage of the specimens during the process of fixing and mounting.

Amphistome Cercariæ.

Three species of amphistome cercariæ were found in my collections. For the first of these I propose the name *Cercaria cortii.*

Of twenty-three specimens of *Planorbis trivolvis* collected at Cherryvale, Kan., October 16, one was infected with *Cercaria cortii.* The snails were found adhering to rocks at a depth of from six inches to a foot in a large pond of clear water.

On October 17 the snails were isolated in individual watch glasses and kept covered with water, the water being changed each day. Cercariæ were noticed in one dish as follows, the observations being made in the morning at eight o'clock:

October 18	6 cercariæ.
October 19	12 cercariæ; all others encysted.
October 20	3 cercariæ; all other cercariæ encysted.
October 21	0 cercariæ; all other cercariæ encysted.
October 22	0 cercariæ; all other cercariæ encysted.
October 23	0 cercariæ; all other cercariæ encysted.
October 24	0 cercariæ; all other cercariæ encysted.
October 25	50 cercariæ; five encysted.
October 26	6 cercariæ; all others encysted.
November 2	All cysts still alive in water.
November 3	One new cercaria encysted.
November 4	One new cercaria encysting.
November 6	One new cercaria; all encysted.
November 5-7	No new developments.
November 8	Snail dead.

The cercariæ within the cysts were alive after having been kept in the water for twenty days, although only those that were opened within four days after they encysted, moved about.

One lot was allowed to dry for forty-eight hours, being kept in a covered watch glass. The air was moist, but there was no water covering the cysts. The cysts were covered with water and later opened. The cercariæ were alive and active. Longer and more complete desiccation proved fatal to the cercariæ.

The process of encysting was carefully noted for this species. The swimming ceases; the anterior end of the body is directed downwards or towards the side of the container; the oral sucker becomes attached; the tail vibrates rapidly, then the cercaria loosens its hold and goes through creeping motions, but does not swim any. This process is repeated a few times, then the cercaria flattens itself against the glass and assumes a spherical shape. The head is twisted from side to side within the cyst wall, and the tail continues to vibrate. The worm becomes more transparent and the cercaria seems to loosen from the outer wall, which assumes a furry appearance, due to the giving off of cystogenous material by the cystogenous glands. At this time, the cercaria begins peculiar rotating movements, which continue from an hour and twenty minutes to two hours and ten minutes. The motion consists of a series of intermittent movements. The time required for a cercaria to make a complete turn varied from one and three-fourths minutes to two and a half minutes, with from thirteen to twenty-two separate movements in a revolution.

The cyst wall becomes thicker and more transparent. Sometimes the tail loosens and swims away, while at other times it may remain loosely attached to the cyst. One tail vibrated constantly for eight hours after the cercaria began to encyst. The tail as well as the body gives off cystogenous material, which is in the form of a delicate sheath surrounding that organ and at a distance of one-half the width of the tail from it.

The motions of the encysting cercaria become slower and slower, and finally cease as the process is completed. When the cyst is fully formed it is much more transparent than the free-swimming cercaria, and the worm is coiled within the cyst with its anterior and posterior ends in contact. Slight spasmodic motions within the cysts can be noted for three or four days after encystment. Sometimes the cercaria breaks out of its cyst by rupturing the wall, and forms a new cyst. The cercariæ usually encysted on the bottom and sides of the watch glass nearest the window, and were shaped like a deep plano-convex lens. In a few cases where encystment took place among masses of snail fæces and

not against the side of the container, the shape of the cysts was ellipsoid.

Cercaria cortii is a rapid swimmer, and its swimming motions are strikingly regular. It would often propel itself in a straight line from one side to the other of a Syracuse watch glass. Creeping motions were rather infrequent in open water, but under the cover slip this was the usual method of locomotion.

The measurements of this cercaria were taken from preparations mounted in glycerine and glycerine jelly after the specimens were killed by heating slightly, and represent as accurately as possible the size of the form in life.

This cercaria is elongate, oval, and wider at the posterior than at the anterior end. It is capable of assuming shapes varying from spherical to long and narrow. The length of the body is .94 mm. and the length of the tail is .87 mm.; a total length of 1.81 mm. The width of the acetabulum is .25 mm. and the width of the oral sucker is .08 mm. The edge of the oral sucker presents a finely lobated appearance.

The mouth is situated within the oral sucker and opens into an oral cavity .01 mm. wide and .06 mm. long. This is separated from the esophagus by a constriction. The esophagus narrows into a pharynx about one-third the diameter of the oral cavity. It is surrounded by a band of longitudinal muscle fibers .027 mm. in outside diameter. The width of the digestive diverticula is .017 mm.

Fig. 2, a cross section through the region of the eyespots, shows the brain. The nerve cords could not be traced any distance from this region.

The anterior one-third of this cercaria is heavily pigmented with dark pigment.

The esophagus is long and narrow. The digestive tract extends five-sevenths of the length of the body. The two eyespots are prominent and are .028 mm. in diameter and .16 mm. apart. The tubes of the excretory system extend from near the eyes to just in front of the acetabulum.

The excretory tubes are made up of large flame cells, irregularly joined together, and very closely associated with the digestive tract. In the living form these tubes appear to be cylindrical and rather regular in shape, but cross sections of the fixed specimens (fig. 3) show the true nature of these ducts. In some places they seem to take a spiral course around the diverticula of the digestive tract. These cells contain concretions .007 mm. in diameter.

In the living form these concretions look like highly refractive cell nuclei and mark out the excretory tract very distinctly. The excretory vesicle into which the paired tubes open is just anterior to the acetabulum and is .017 mm. in diameter.

From the living specimen one would think that the tail contains an excretory canal, but cross sections show that the central region of the tail is made up of very large cells with extremely delicate walls. Encircling this central region is a cylinder of smaller cells and a narrow band of outer longitudinal muscle fibers.

The anlagen of the reproductive organs show as rounded masses of deeply staining cells, within the diverticula of the digestive tract. Cystogenous glands are present all over the body excepting in the immediate vicinity of the oral sucker and mouth. Within the cystogenous glands are granules of the cyst-forming material.

Of twenty-three specimens of *Planorbis trivolvis* collected at Lawrence, Kan., October 10, one was infected with *Cercaria diastrophora* Cort. The following additional description and accompanying plates may be considered merely supplemental to Cort's description of this form. These studies were made before the identification of the species had been established as being the same as *C. diastrophora* Cort.

In general appearance this form corresponds to *Cercaria cortii*, but it is much smaller, measuring .48 mm. long and .2 mm. wide. The tail is .58 mm. long. The oral sucker is small, measuring .06 mm. in width, while the acetabulum is exceedingly large, measuring .15 mm. in outside diameter.

The esophagus is very slender, opening into a bulbous pharynx three-fourths as broad as the tip of the oral sucker. The eyespots are prominent and are oval rather than spherical, measuring .027 mm. by .036 mm. They are separated by a distance equal to the long axis of the eye.

The excretory system consists of paired ducts opening into an excretory vesicle anterior to the acetabulum. This vesicle also receives smaller ducts from the region of the acetabulum. The excretory pore opens on the dorsal surface.

This species stains deeply with hæmalum, excepting in the region of the oral sucker, where the cells seem to be devoid of nuclei. The tail is made up of large central cells surrounded by a ring of smaller cells. It contains no excretory canals.

The immature cercariæ of this species are blunt-tailed and clumsy. No internal organs can be distinguished excepting the eyespots, which are rudimentary.

The rediæ of this form are short and blunt and are provided with anterior and posterior locomotor projections. The digestive tract is short and blunt. The cercariæ within the rediæ were all immature, due perhaps to the fact that the snail was not fixed until cercariæ had ceased to emerge from it. The rediæ average .88 mm. long and .25 mm. wide.

Of twenty specimens of *Planorbis trivolvis* collected at Lawrence, Kan., July 22, one was parasitized with *Cercaria inhabilis* Cort. These snails were obtained from the surface of Horseshoe lake, where they were attached to twigs and floating objects.

The cercariæ emerged from the snails that had been isolated in a watch glass, and remained in the immediate vicinity of the snail. Owing to their heavy tails and unwieldy bodies, their attempts at swimming resulted in their floundering about. The creeping motions also were very feeble. When the snail was crushed, rediæ and cercariæ in various stages of development were found in the liver. A remarkable fact in connection with this species was that the rediæ were smaller than the cercariæ.

This apparent inconsistency may be explained by comparing the free cercariæ with the sections of the infected snail liver, where free cercariæ are to be found completing their development in the digestive gland near the periphery of that organ.

The length of this species is .76 mm. and the width is .23 mm. There are two prominent pigment spots on the anterior dorsal surface of the body. The digestive tract consists of a slender pharynx leading from the mouth, situated within the oral sucker, to the diverticula of the intestine. The excretory system consists of paired tubes opening into the excretory pore just anterior to the ventral sucker. A mass of deeply staining cells, the anlage of the reproductive organs, is anterior to and below the pore.

The rediæ are elongated sacs, the immature ones averaging .55 mm. long and .07 mm. wide. The mature ones are slightly longer and twice as wide. The immature forms contain only germ balls, while the mature ones contain both germ balls and developing cercariæ that move about within the walls. No mature cercariæ were observed within the rediæ of this species.

Distome Cercariæ.

In my material are nine distome cercariæ, belonging to five subgroups.

MEGALUROUS CERCARIÆ.

Of twenty specimens of *Planorbis trivolvis* collected at Lawrence, Kan., July 27, one was infected with *Cercaria magnacauda*. This is an active free-swimming form, positively phototropic. It did not undergo creeping motions excepting when the tail was detached from the body.

The behavior of this cercaria was noted carefully. They would swim actively for a few minutes, then come to rest, pointing head downwards at an angle of fifteen degrees from the vertical. In this position the tail is blade shaped and flattened dorsoventrally. Practically all of the cercariæ would be either resting or swimming at any one time. These cercariæ were especially virile, living from thirty to forty-eight hours after emerging from the snail. The tail invariably became detached from the body and continued swimming after the death of the former. This form did not encyst.

The rediæ of this species were found in tangled masses in the liver of the host. They average .9 mm. long and from .1 mm. to .5 mm. wide, having a short digestive tract. The mouth is terminal and well defined, being provided with a large median stylet.

This remarkable cercaria has a total length of 1.56 mm., the body comprising only .127 mm. leaving it less than one-eleventh of the length of the tail. These measurements, which were taken from balsam mounts, are approximately normal, excepting that the tail is somewhat flattened.

From a study of the living material only the suckers and the concretions in the excretory ducts could be seen. Mounted specimens show the enormous excretory tract and the reproductive anlagen, while the cephalic glands can be seen in sections.

The excretory tract consists of two tubes extending from near the oral sucker to a large excretory bladder, posterior to the ventral sucker. This bladder communicates with another in the anterior narrow part of the tail. Opening into this secondary bladder is the median excretory duct of the tail, which is ventral in position.

The reproductive anlagen appear as two masses of cells anterior and posterior to the ventral sucker. No digestive tract could be traced.

The oral sucker is .007 mm. in outside diameter, while the ventral sucker is twice this size. The tail is made up of large parenchymous cells and longitudinal and cross-muscle fibers. Owing to its delicate structure it could not be embedded for sectioning.

ECHINOSTOME CERCARIÆ.

Of one hundred specimens of *Physa gyrina* collected at Lawrence, Kan., August 19, one was infected with *Cercaria fusiformis*. This species is characterized by a symmetrical spindle-shaped body with a long digestive tract extending from the oral sucker to near the caudal end of the body. While this species had all of the characteristics of the echinostome cercariæ, no spines are present anywhere. The region of the oral sucker presents a collared appearance. The total length of this cercaria is .77 mm. and the width is .07 mm. The oral sucker has an external diameter of .028 mm. and an internal diameter of one-fourth that size. The ventral sucker is about the same size and is capable of being greatly extended or projected. This form used the ventral sucker extensively in holding fast to the substratum.

The digestive diverticula do not inclose the ventral sucker as one would infer from the dorsal aspect, but are dorsal to it in lateral view. The digestive tract consists of a very narrow esophagus leading from the mouth to the pharynx. Just back of the pharynx is a median intestine extending as far as the anterior end of the ventral sucker, where it branches into two diverticula that extend back to the posterior end of the body. The esophagus measures .003 mm. wide and .03 mm. long. The width of the pharynx is .018 mm. and that of the intestine .012 mm.

The excretory tract consists of narrow paired tubes extending from the region of the pharynx to the posterior end of the body. The excretory bladder and the excretory duct, in the anterior part of the tail, could not be traced definitely, but appeared as more transparent areas in the toto mount.

The anlage of the reproductive organs is a dense mass of cells near the posterior end of the body.

The posterior sucker is very muscular. The figures accompanying the plate show this sucker open, closed, and extended.

The rediæ are large and contain from six to eight mature cercariæ, together with a few large germ balls. They are distended in places by the cercariæ contained within. The average measurements of the rediæ are, length 1.91 mm. and width .13 mm. to .33 mm.

GYMNOCEPHALOUS CERCARIÆ.

Of fifty specimens of *Physa integra* collected at Chanute, Kan., October 17, two were infected with *Cercaria gracilis*. I propose this name because of the ability of the species to draw itself out

into a very slender shape. The infection was slight, and these studies were made entirely from the living material and two mounted specimens.

This cercaria is exceedingly active and is capable of extending the body until it is as narrow as the tail. This form can also extend the ventral sucker until it appears prominent in a lateral view. In a characteristic position this form is slender, heart-shaped. The oral sucker is minute, the ventral sucker being four times as large.

The esophagus is slender and has a fold near the anterior end. Just beneath the fold is the narrow pharynx.

The diverticula of the digestive tract are broad, encircling the ventral sucker. The excretory system could not be made out, but paired rows of cells with highly *refractive* nuclei, or possibly concretions, mark this tract. The tail is broad and large, being one-third the width of the body and a little over twice the length of the same. The entire length of this cercaria is .53 mm.

The rediæ are of unusual shape, tapering at both ends with a definite collar near the anterior end. The digestive tract is slender, extending about half the length of the body. Within the rediæ are germ balls, developing cercariæ, and mature cercariæ. The dimensions of the rediæ are 1.6 mm. long and .33 mm. wide in the widest place.

FURCOCERCOUS CERCARIÆ.

Two per cent of large numbers of *Physa gyrina*, 436 in all, collected at Lawrence, Kan., during the months of July and August, from Haskell pond and the lake at Lakeview, were parasitized with a form for which I propose the name *Cercaria inversa*, because of its being directed tail foremost in swimming. The cercariæ emerged freely from the snails and flitted about rapidly in the water with a peculiar vibratile motion, directed tail foremost. Creeping movements were not observed excepting under the cover slip, when the tail became severed from the body. When the parasitized snails were crushed, rediæ containing cercariæ in all stages of development, from mere germ balls to mature forms, were present. The various stages in the development of the cercariæ could be easily observed because of the characteristics of the tails, which varied in length from mere stubs and rounded lobes to the elongate bifurcated tails of the mature forms.

The rediæ* were in a tangled mass in the liver. The largest

* Further studies showed that the rediæ of this species may be four times as long as the one shown in figure 47, and much constricted, resembling link sausage.

ones exhibited a slightly waving motion. Under the cover slip cercariæ emerged from the rediæ, usually tail first, from the birth pore near the anterior end. It required about two minutes for a cercaria to free itself from the redia. This form did not encyst, but the cercariæ soon died in the water, in no case living more than four hours after emerging from the snail. Those liberated when the snail was crushed lived only from eight to twelve minutes. The number of this species emerging from a single snail was estimated at five thousand.

Cercaria inversa is a small furcocercous form, corresponding as to size, shape and behavior to *Cercaria douthitti* described by Cort (1915), from *Lymnaea reflexa*. This species, however, contains no eyespots and is found in rediæ instead of sporocysts. The length of the body is .16 mm. in well-extended specimens, and the width is .045 mm. The unbranched part of the tail has a length of .26 mm. and a width of .027 mm., while the lobes are five-sixths as long as the main part of the tail and one-half as wide. These lobes taper to rather a sharp point. The openings in the suckers are about the same size, .01 mm., but the outside dimensions of the oral sucker are greater than those of the ventral sucker. The measurements are, oral sucker .042 mm. and ventral sucker .028 mm.

As in *Cercaria douthitti*, the region back of the center is filled with large cephalic glands. The ducts of these glands extend forward and open alongside the oral sucker (fig. 50). The excretory system of the body region could not be traced, but a duct extends through the main part of the tail and is joined by ducts from each branch. An excretory pore opens to the exterior between the forks of the tail.

The anlage of the reproductive organs is a mass of small cells near the posterior end of the body and ventral in position.

Six per cent of large numbers of *Physa gyrina* collected at Lakeview, Kan., August 20, were infected with a form for which I propose the name *Cercaria echinocauda*. Collections of snails from the same locality, made October 12, showed two per cent to be infected.

This cercaria was very active, swimming both forwards and backwards, but usually forwards, with a vibratile motion of the tail. The tail was loosely attached to the body and was easily severed. When this occurred the body died in about twenty minutes, while the tail lived for two hours, swimming about

actively. Six hours was the maximum time that these cercariæ remained alive after emerging from the snail.

The body of this species is .31 mm. long and .125 mm. wide. The main part of the tail has a length of .54 mm., while the branches are .18 mm. long. Two large eyespots are present. They are made up of minute pigment granules and measure .02 mm. by .014 mm. in dorsal view, being longer in the transverse direction.

An excretory tube begins in the branches of the tail and extends throughout the length of that organ to the excretory pore in the posterior end of the body. The excretory tract could not be made out in the body region.

The branches of the tail are provided with a sort of fin extending around the tip. This fin is beset with minute spines.

A mouth is present in the oral sucker, but there is no digestive tract leading from it.

The anlage of the reproductive organs is ventral in position and near the posterior end of the cercaria.

A peculiar characteristic of this species was that in the fixed specimen the tail is usually bent sharply at its junction with the body.

The rediæ of this species average .2 mm. long and .11 mm. wide. They are filled with germ balls and cercariæ in various stages of development. The anterior end is somewhat pointed, while the posterior end is rounded.

Cercaria echinocauda is similar to *Cercaria ocellata* La Valette St. George, as the measurements for this species fall within the wide range described for *Cercaria ocellata*. Both forms are provided with fin-like projections on the tail. The tail of *Cercaria echinocauda* is not very contractile, contrasting with that of *Cercaria ocellata*. The oral sucker of *Cercaria echinocauda* is also much larger than that of *Cercaria ocellata*. *Cercaria echinocauda* develops in rediæ, while *Cercaria ocellata* is found in sporocysts.

Of thirteen specimens of *Planorbis trivolvis* collected at Lawrence, Kan., October 7, two were infected with a cercaria for which I propose the name *Cercaria quieta*, because of its often remaining motionless, floating in the water for brief periods of time. Like *Cercaria inversa*, this cercaria has a bifurcated tail and swims by means of a rapid vibratile motion of this organ.

The tail is enormous in size compared to the body and is not constricted off from the body in the living specimen as is the case with other furcocercous forms. The tail of this species is never

severed from the body in the living form, and only rarely did it become lost during mounting.

The cercaria can swim either forwards or backwards. The tips of the bifurcated tail are fitted with adhesive organs by means of which they attach themselves to the substratum or other cercariæ. Both oral and ventral suckers are small and of uniform size, measuring .027 mm. in diameter.

The excretory system consists of paired ducts leading from the anterior end of the body to the junction of the body and tail, where they anastomose and extend on back to the excretory pore which opens between the forks of the tail. No excretory vesicle is present but the excretory tube is somewhat dilated just anterior to the excretory pore.

The anlage of the reproductive system is posterior to the ventral sucker. No digestive tract could be traced.

The total length of the cercaria is .8 mm. and the width is .08 mm., the main part of the tail being as wide as the body. Of the total length the body makes up one-fifth, the unbranched tail two-fifths, and the branched tail two-fifths.

The redia of this species is long and cylindrical, measuring 1.52 mm. by .2 mm. Each redia contains many cercariæ, about one-fifth of which are mature. The rediæ were so tangled in the liver that it was almost impossible to dissect them out entire.

XIPHIDIO CERCARIÆ.

My material contains three species of xiphidio cercariæ. Owing to the extreme difficulty of studying these small forms, my descriptions are in some places incomplete. I am unable to make these species fit in with previously described forms, much as they resemble forms described by Cort and Luhe.

For the first of these species I propose the name *Cercaria haskelli*, from the locality where it was found (Haskell pond). One of thirty-three specimens of *Physa gyrina* collected from this locality at Lawrence, Kan., July 12, was infected with this species.

Cercaria haskelli is a rapid swimmer, the swimming alternating with creeping movements. A marked characteristic of this species was that it could extend the tail until it was three or four times the length of the body. When this form was swimming, the body would be contracted into a ball and the tail would be very much extended.

The measurements for this species in an average state of contraction are, length .15 mm. and width .05 mm. The tail meas-

ures four-fifths the length of the body. A stylet protrudes from the region of the oral sucker. This stylet measures .037 mm. long and has a width at its base of one-fifth of the length. It tapers to a point and has no enlargements anywhere along its length.

The oral sucker is .04 mm. in diameter and the ventral sucker measures .03 mm. In longitudinal section, both suckers open into pouches wider than the external openings. There are two layers of cuboidal cells in the wall of the ventral sucker.

The muscular layers comprising the outer wall of the cercaria average .003 mm. in thickness.

The esophagus is exceedingly narrow, averaging .0015 mm. in diameter. It is grounded by a ring of deeply staining cells corresponding to the muscular pharynx seen in other forms. The esophagus broadens into a median digestive tract .03 mm. long and .0125 mm. wide.

Anterior to and dorsal to the ventral sucker are numerous unicellular glands. These are probably stylet glands, as their position is different from that of the cephalic glands described for *Cercaria inversa*, for instance. No ducts could be found leading from these glands.

The only excretory tract found consists of irregular spaces lying against the dorsal wall of the ventral sucker. In transverse section of the tail, four large central cells can be seen surrounded by a ring of muscle fibers. In some places the walls between these central cells are made up of smaller narrow cells.

The anlagen of the reproductive organs are in two masses dorsal to the ventral sucker. These masses are connected by a narrow band of similar cells.

Cercaria haskelli is found in sporocysts. They are rounded elongate sacs containing germ balls, developing cercariæ and mature cercariæ. The sporocysts are from .25 mm. to .42 mm. long and are from one-third to one-half as wide.

Of twenty-three specimens of *Planorbis trivolvis* collected at Cherryvale, Kan., October 16, five were infected with *Cercaria gregaria*. The cercariæ emerged from the snails by thousands and had the peculiar habit of massing together in the water. They would lose their tails and form such compact masses that they could not be separated without tearing the tissues apart. These masses contained from fifty to five hundred individuals each. The cercariæ remained alive in this condition for eight hours. No encystment was seen, and very few cercariæ remained for any length of time without joining with one of the masses.

This is an exceedingly minute form, measuring only .37 mm. long, including the tail, which is half the length of the body. The width is .03 mm. Both of the suckers are the same size, averaging .015 mm. in outside diameter. This form has a stylet .006 mm. long. On account of the minuteness of this form, the internal structures could not be made out clearly. The best results were obtained by staining intravitally with picrocarmine. With this stain paired masses of cephalic glands with their ducts could be made out. The tail of this form consists of large cells with a definite single row of nuclei, showing prominently in the median line.

Sectioning the snails from which these cercariæ emerged failed to reveal either sporocysts or rediæ.

Seventy-five per cent of hundreds of *Planorbis trivolvis* collected at Pratt, Kan., August 22, were infected with a small xiphidio cercaria which I propose to call *Cercaria kansiensis*. The body of this form averages .06 mm. wide and .09 mm. long. The tail in an average state of contraction is .064 mm. long.

The oral sucker is .024 mm. in diameter and the ventral sucker is .028 mm. The openings in both suckers are .007 mm. in diameter. There is a bicornuate groove in the posterior end of the body into which the tail fits.

No digestive tract could be traced, but a ring of deeply staining cells marks the region of the pharynx.

Large unicellular cephalic glands are present. They number about four on a side. No ducts could be found leading from them.

No excretory tubes could be found, but there is a large excretory bladder .01 mm. wide and .016 long, posterior to the ventral sucker. The long axis of this bladder is in a transverse direction to the long axis of the body.

The anlagen of the reproductive organs consist of two masses of cells dorsal to the ventral sucker. The posterior mass is the larger.

Special studies were made of the stylet of *Cercaria kansiensis*. It is embedded in the muscles of the thick-walled oral sucker, dorsal to the mouth opening, and can be withdrawn into a hollow receptacle. Two camera-lucida sketches (figs. 57 and 58) show the stylet extended and contracted. The stylet measures .02 mm. in length. At the base and near the point it has a width of one-sixth its length, but between these points it is narrower.

Cercaria kansiensis is found in sporocysts averaging .33 mm. long and about one-third as wide.

In all cases where this form was found, the infection was heavy, the liver of the snail being filled with almost a solid mass of the sporocysts. Estimates of the numbers of cercariæ emerging from any one snail ran from five thousand to eight thousand.

Notes.

The following notes, while adding little to this paper, might be of assistance to any one wishing to work out life histories of trematodes.

The collecting grounds from which my material was taken covered a wide range of habitats, from temporary pools and pasture streams to artificial ponds and permanent lakes.

Only two genera of snails were examined. Infection was very rarely found in young snails, and never in snails collected from temporary pools or pasture streams. Old ponds harboring fish, frogs, muskrats and water snakes usually contained infected snails.

All of the furcocercous cercariæ were found in *Physa* collected from muddy permanent ponds. The large amphistome cercariæ and the xyphidio cercariæ were found in the genus *Planorbis* in the larger, clearer lakes. Lakes of this type are inhabited by the species of water life mentioned for the other type of ponds, and in addition are frequented by migratory birds.

The heaviest infection was found the latter part of August at the State Fish Hatchery at Pratt, Kan., where the number of snails parasitized ran as high as 90 per cent.

Only one of the species studied, *Cercaria cortii*, encysted under observation, and no cysts of any of the other forms were found outside of the snail.

One specimen of *Planorbis trivolvis*, collected from pond No. 5, July 24, showed the presence in the digestive gland of large numbers of spherical cysts about .05 mm. in diameter. These cysts correspond to those of *Cercaria trivolvis* described by Cort as having been found in the body cavity of *Planorbis trivolvis*.

One characteristic of *Cercaria gregaria* possibly throws some light on the life history of the species. This is the peculiar habit that the cercariæ have of losing their tails and forming compact masses in the water, each mass containing from fifty to five hundred individuals.

The following tables will be found useful for making summaries concerning the amount and kind of infection:

Pond Index.

No.	
1–Lawrence, Kan	Haskell, east of dairy barn.
2–Lawrence, Kan	Haskell, drainage ditch, south.
3–Lawrence, Kan	Stubbs' pond, northwest campus.
4–Lawrence, Kan	Stream, Woodland Park.
5–Lawrence Kan	Horseshoe Lake, six miles southeast.
6–Lawrence, Kan	Stream northwest of Griesa nursery.
7–Lakeview	East end of road through lake.
8–Lawrence Kan	Stream near E. A. Richards', six miles northwest.
9–Lawrence Kan	Stream one-half mile southwest of stream No. 8.
10–Lawrence, Kan	Bismarck Grove, North Lawrence.
11–Lawrence, Kan	Lake north of U. P. depot.
12–Lakeview	East of depot.
13–Pratt, Kan	State Fish Hatchery, Pond No. 22.
14–Pratt, Kan	State Fish Hatchery, Pond No. 39.
15–Pratt, Kan	State Fish Hatchery, Pond No. 41.
16–Pratt, Kan	State Fish Hatchery, Pond No. 51.
17–Pratt, Kan	State Fish Hatchery, Pond No. 77.
18–Pratt, Kan	State Fish Hatchery, Pond No. 1.
19–Lawrence, Kan	Railroad, one-half mile southeast of Haskell.
20–Lawrence, Kan	Pond on East Fifteenth street.
21–Baldwin, Kan	Pond in east edge of town.
22–Baldwin, Kan	Pond southwest of depot.
23–Baldwin, Kan	Pond center of town.
24–Ottawa, Kan	Stream east of Park, near library.
25–Ottawa, Kan	Pond near race track.
26–Ottawa, Kan	River west of west bridge.
27–Chanute, Kan	Santa Fe, one-half mile north of depot.
28–Chanute, Kan	Santa Fe, north of Frisco crossing.
29–Chanute, Kan	Santa Fe, two miles north, west side.
30–Cherryvale, Kan	Lake in city limits.
31–Cherryvale, Kan	Pond one-half mile north, along Santa Fe.
32–Abilene, Kan	Engle's pond, seven miles southwest.
33–Abilene, Kan	Pond one-half mile east of R.C. Lahr, southwest.
34–Abilene, Kan	Stream four and one-half miles southwest.

Percentage of Infected Snails.

Date.	Pond.	Host.	Number of snails.	Number infected.	Species.
7- 7	1	*Physa gyrina*	25	2	*C. inversa.*
7- 7	2	*Physa gyrina*	20	1	*C. haskelli.*
7- 9	1	*Physa gyrina*	95	0	———
7- 9	3	*Physa gyrina*	50	0	———
7-10	1	*Physa gyrina*	20	0	———
7-12	1	*Physa gyrina*	33	6	*C. inversa.*
7-20	1	*Physa gyrina*	130	0	———
7-21	4	*Physa gyrina*	23	0	———
7-22	1	*Physa gyrina*	30	1	*C. inversa.*
7-24	5	*Planorbis trivolvis*	15	1	*C. magnacauda.*
7-31	6	*Physa gyrina*	6	0	———
7-31	8	*Physa gyrina*	5	0	———
7-31	9	*Physa gyrina*	5	0	———
8- 2	7	*Physa gyrina*	10	1	*C. inversa.*
8- 3	7	*Physa gyrina*	12	2	*C. inversa.*
8- 4	8	*Physa gyrina*	20	0	———
8- 4	9	*Physa gyrina*	10	0	———
8-18	10	*Physa gyrina*	10	0	———
8-18	11	*Physa gyrina*	12	0	———
8-19	1	*Physa gyrina*	100	1	*C. fusiformis.*
8-20	12	*Physa gyrina*	100	6	*C. echinocauda.*
8-28	13	*Planorbis trivolvis*	16	4	*C. kansiensis.*
8-28	14	*Planorbis trivolvis*	30	13	*C. kansiensis.*
8-28	15	*Planorbis trivolvis*	20	3	*C. kansiensis.*
8-28	16	*Planorbis trivolvis*	20	16	*C. kansiensis.*
8-28	17	*Planorbis trivolvis*	20	16	*C. kansiensis.*
8-29	18	*Planorbis trivolvis*	29	13	*C. kansiensis.*
8-29	17	*Planorbis trivolvis*	25	21	*C. kansiensis.*
				2	*C. inversa.*
8-30	16	*Planorbis trivolvis*	50	45	*C. kansiensis.*
8-31	17	*Planorbis trivolvis*	50	34	*C. kansiensis.*
				2	*C. inhabilis.*
8-31	16	*Planorbis trivolvis*	90	75	*C. kansiensis.*
10- 7	12	*Physa gyrina*	50	1	*C. echinocauda.*
10- 7	5	*Planorbis trivolvis*	13	2	*C. inhabilis.*
10- 8	19	*Planorbis trivolvis*	12	2	*C. inhabilis.*
10-12	19	*Planorbis trivolvis*	23	1	*C. inhabilis.*
10-19	21	*Physa gyrina*	50	0	———
10-19	22	*Physa gyrina*	50	2	*C. haskelli.*
10-19	23	*Physa gyrina*	50	0	*C. haskelli.*
10-19	25	*Physa gyrina*	50	2	*C. haskelli.*
10-19	26	*Physa gyrina*	50	0	———
10-19	27	*Physa gyrina*	50	0	———
10-19	28	*Physa integra*	50	2	*C. gracilis.*
10-19	29	*Physa gyrina*	50	0	———
10-20	30	*Physa gyrina*	50	7	*C. gregaria.*
10-21	30	*Physa gyrina*	27	5	*C. cortii.*
11- 9	31	*Physa gyrina*	50	0	———
11- 9	32	*Physa gyrina*	100	0	———
11- 9	33	*Physa gyrina*	25	0	———

Favorable circumstances enabled the writer to continue his investigations of the cercariæ described in this paper during the summer and fall of 1916, and as late as January 5, 1917. The vicinities of Lawrence and Pratt were resurveyed, with the results that the species found the preceding year were verified by repeated collections and comparisons. *Cercaria magnacauda* was not found this season.

Many cases of double infection were found in *Physa gyrina* from pond No. 1. These cases of double infection were confined to two groups, one consisting of an infection of *Cercaria inversa* in rediæ in the digestive gland, and unidentified cysts in the body cavity,

corresponding in size to those of *Cercaria trivolvis* Cort. The other group consisted of a combination of the above-named cysts in the digestive gland, and a cluster of much larger cysts of a polystome cercaria on the periphery of the middle part of the snail inside of the shell.

Experimental work carried on with *Planorbis trivolvis* infected with *Cercaria kansiensis* at Pratt, during the summer of 1916, showed that cercariæ were continually emerging from snails kept in wire cages suspended in the ponds at the Fish Hatchery. These snails were taken from the cages each morning and confined in watch glasses in the laboratory. No characteristic swarming was noticed, but instead a few cercariæ could be seen emerging from the snails every time they were examined.

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EXPLANATION OF PLATES.

With the exception of Plate VII, all figures are drawn with the camera lucida. The abbreviations are after the plan adopted by Cort.

ABBREVIATIONS USED.

ac–acetabulum.
bp–birth pore of redia.
br–brain.
c–concretions.
cg–cystogenous glands.
ccg–cephalic glands.
dc–ducts of ccg.
e–eyespot.
es–esophagus.
ex–excretory system.
exp–excretory pore.
exd–excretory duct.
exv–excretory vessel.
gb–germ ball.
i–intestinal cæcum of cercaria.
ir–intestine of redia.
la–locomotor appendage of redia.
lc–large central cells of tail.
ml–muscle layer.
os–oral sucker.
p–pigmentation.
pb–pharyngeal bulb.
pr–pharynx of redia.
ra–reproductive anlage.
s–stylet.
sr–stylet of redia.
sc–cercariæ in sporocysts.
sg–stylet glands.
sw–wall of sporocyst.
vs–ventral sucker.

PLATE I.

FIG. 1. *Cercaria cortii.* × 61.
FIG. 2. Cross section of *C. cortii* through brain. × 122.
FIG. 3. Cross section of *C. cortii* through reproductive anlage. × 122.
FIG. 4. Cross section of tail of *C. cortii.* × 244.
FIG. 5. Longitudinal section of tail of *C. cortii.* × 244.
FIG. 6. Cyst of *C. cortii,* dorsal view. × 61.
FIG. 7. Cyst of *C. cortii,* lateral view. × 61.

PLATE II.

FIG. 8. *Cercaria diastrophora* Cort. × 144.
FIG. 9. Cross section of *C. diastrophora* through oral cavity. × 288.
FIG. 10. Cross section of *C. diastrophora* through pharynx. × 144.
FIG. 11. Cross section of *C. diastrophora* through reproductive anlage. × 144.
FIG. 12. Cross section of tail of *C. diastrophora.* × 288.
FIG. 13. Immature redia of *C. diastrophora.* × 144.
FIG. 14. Mature redia of *C. diastrophora.* × 72.
FIG. 15. Longitudinal section through excretory pore of *C. diastrophora.* × 576.

PLATE III.

FIG. 16. *Cercaria inhabilis* Cort, dorsal view. × 44.
FIG. 17. Immature *Cercaria inhabilis.* × 36.
FIG. 18. Immature redia of *C. inhabilis.* × 36.
FIG. 19. Mature redia of *C. inhabilis.* × 36.
FIG. 20. Cross section of *C. inhabilis* through intestinal cæca. × 160.
FIG. 21. Cross section of *C. inhabilis* through excretory pore. × 160.
FIG. 22. Cross section of tail of *C. inhabilis* near body. × 160.
FIG. 23. Cross section of tail of *C. inhabilis* near posterior end. × 160.
FIG. 24. *Cercaria gracilis.* × 160.
FIG. 25. Redia of *Cercaria gracilis.* × 36.

PLATE IV.

FIG. 26. *Cercaria fusiformis*, dorsal view. × 72.
FIG. 27. *C. fusiformis*, ventral sucker closed. × 160.
FIG. 28. *C. fusiformis*, ventral sucker open. × 160.
FIG. 29. *C. fusiformis*, ventral sucker lateral view. × 160.
FIG. 30. *Cercaria magnacauda*. × 72.
FIG. 31. Body of *C. magnacauda*. × 260.
FIG. 32. *Cercaria quieta*. × 72.
FIG. 33. Ventral sucker of *C. quieta*. × 260.
FIG. 34. Mouth of redia of *C. magnacauda*, longitudinal section. × 260.
FIG. 35. Mouth of redia of *C. magnacauda*, cross section. × 260.
FIG. 36. Redia of *C. fusiformis*. × 30.
FIG. 37. Redia of *C. quieta*. × 36.
FIG. 38. Redia of *C. magnacauda*. × 104.

PLATE V.

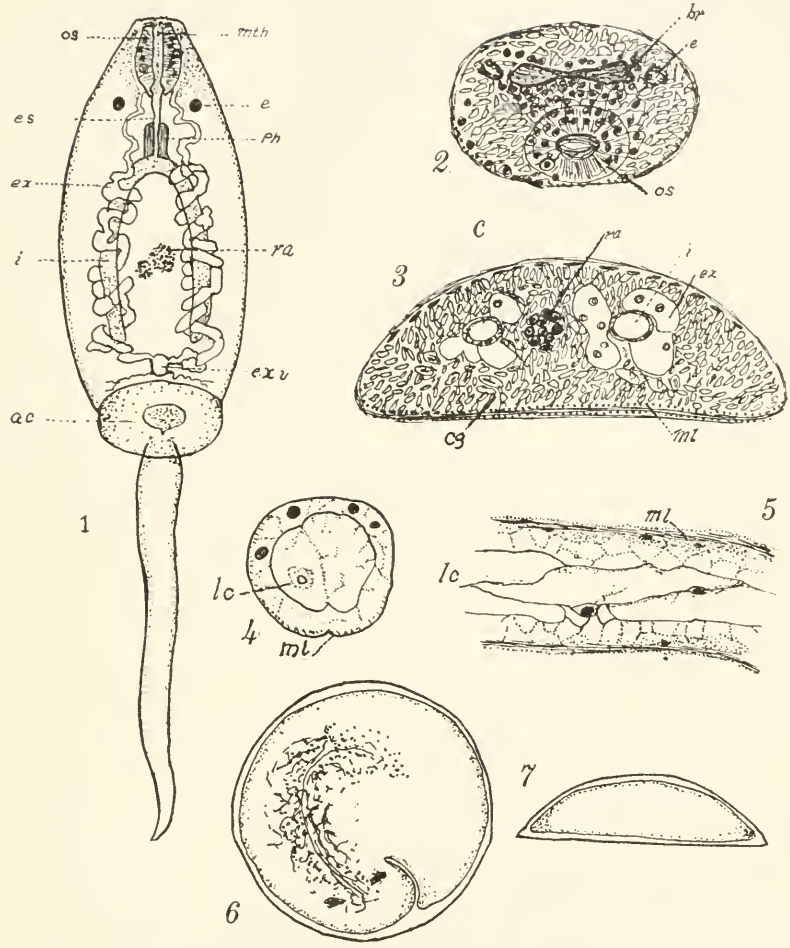
FIG. 39. *Cercaria echinocauda*. × 72.
FIG. 40. *Cercaria echinocauda*. × 72.
FIG. 41. Redia of *C. echinocauda*. × 36.
FIG. 42. Longitudinal section through *C. echinocauda*. × 160.
FIG. 43. Cross section through *C. echinocauda*. × 160.
FIG. 44. Tail of *C. echinocauda*, longitudinal section. × 160.
FIG. 45. Tail of *C. echinocauda*, cross section. × 160.
FIG. 46. *Cercaria inversa*. × 72.
FIG. 47. Redia of *C. inversa*. × 72.
FIG. 48. Cross section of tail of *C. inversa*. × 144.
FIG. 49. Cross section through cephalic glands, *C. inversa*. × 144.
FIG. 50. Cross section through oral sucker, *C. inversa*. × 520.
FIG. 51. Longitudinal section through excretory pore, *C. inversa*. × 260.

PLATE VI.

FIG. 52. *Cercaria haskelli*, dorsal view. × 144.
FIG. 53. *C. haskelli*, longitudinal section. × 160.
FIG. 54. *C. haskelli*, cross section through tail. × 160.
FIG. 55. Sporocysts of *C. haskelli*. × 72.
FIG. 56. *Cercaria kansiensis*. × 260.
FIG. 57. Lateral view of stylet, *C. kansiensis* withdrawn. × 260.
FIG. 58. Lateral view of stylet extended, *C. kansiensis*. × 260.
FIG. 59. Sporocyst of *C. kansiensis*. × 72.
FIG. 60. *Cercaria gregaria*. × 144.
FIG. 61. Longitudinal section of snail liver infected with *C. kansiensis*. × 44.
FIG. 62. Sporocyst *C. kansiensis*, cross section. × 260.

PLATE VII.

Reconstructions of the cercariæ described in the preceding pages all drawn to scale × 36. The numbers refer to the indices accompanying plates I-VI. Numbers 8′ to 56′ refer to sporocysts and rediæ corresponding to the cercariæ.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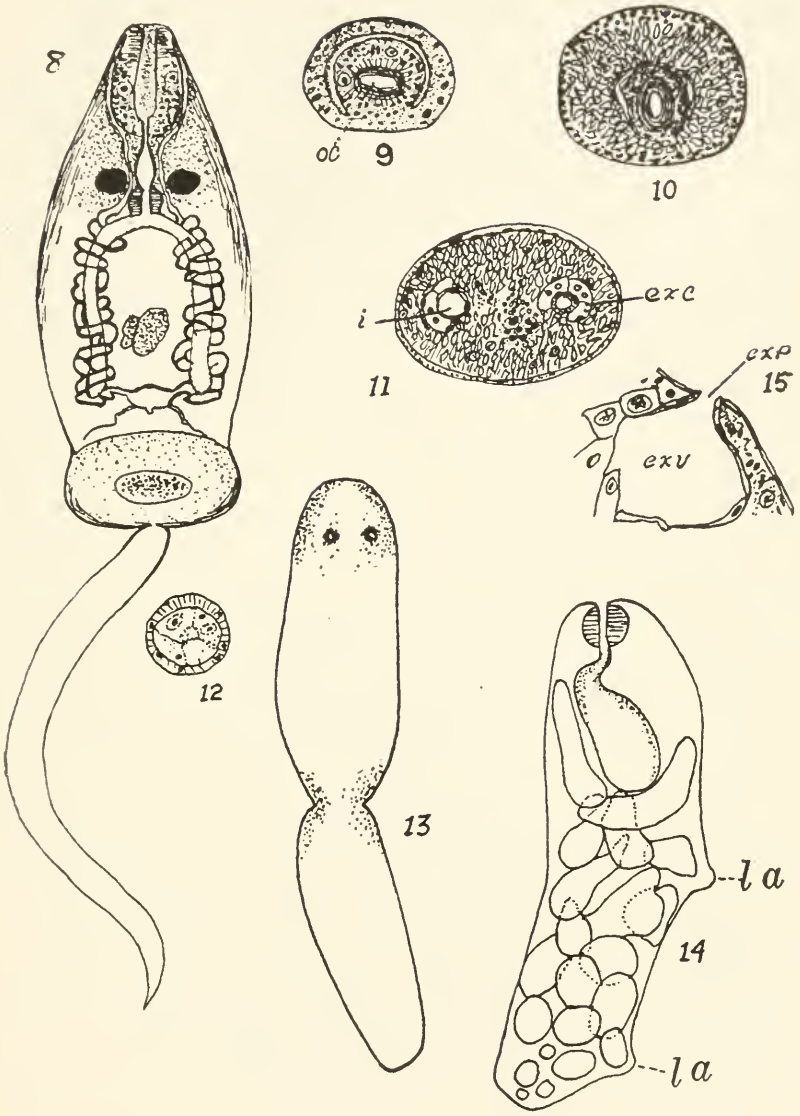
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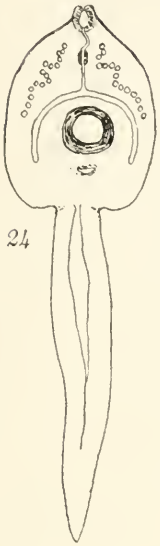
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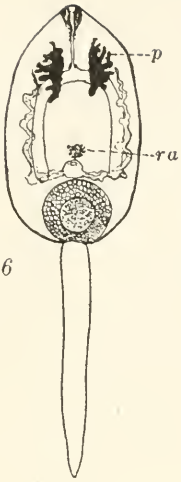

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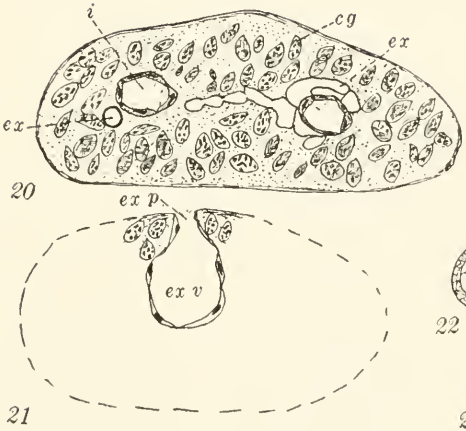

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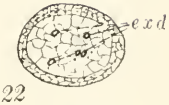

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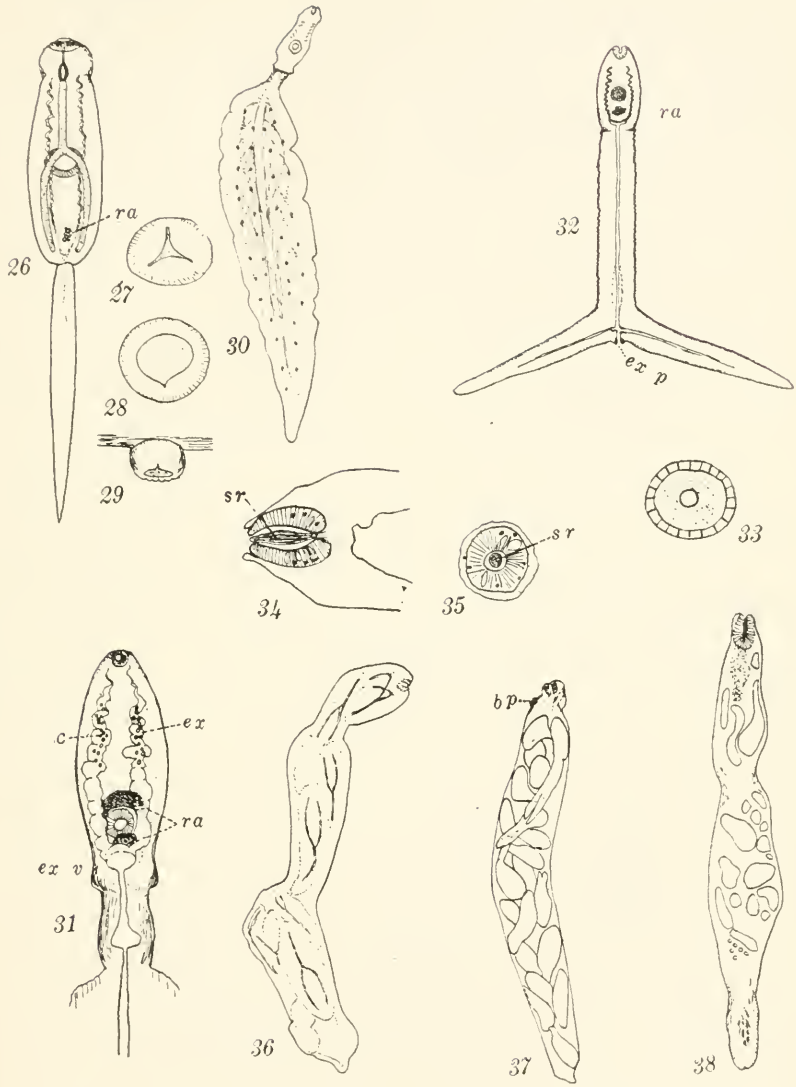
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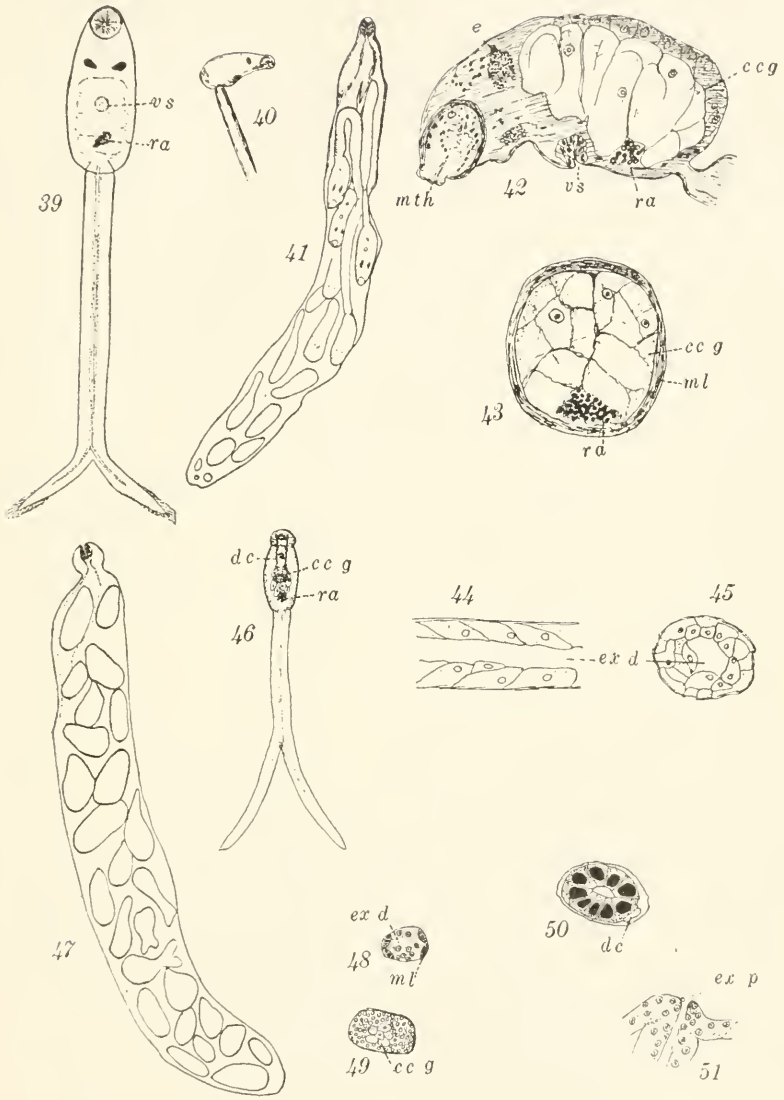
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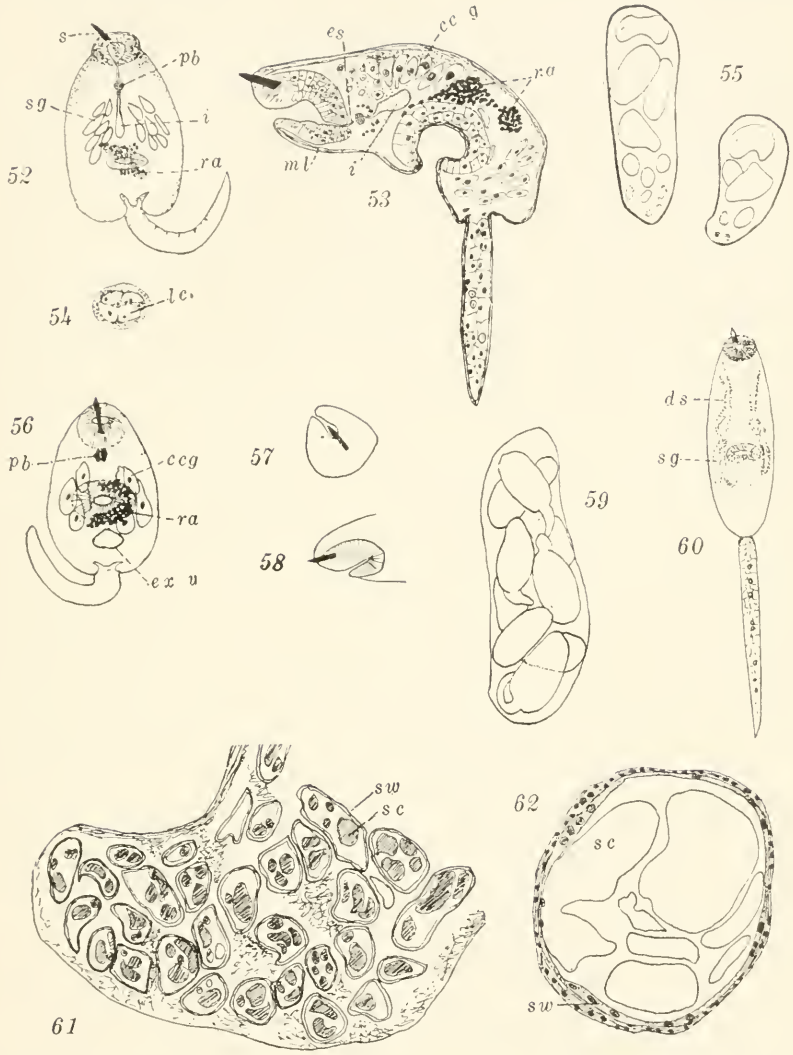
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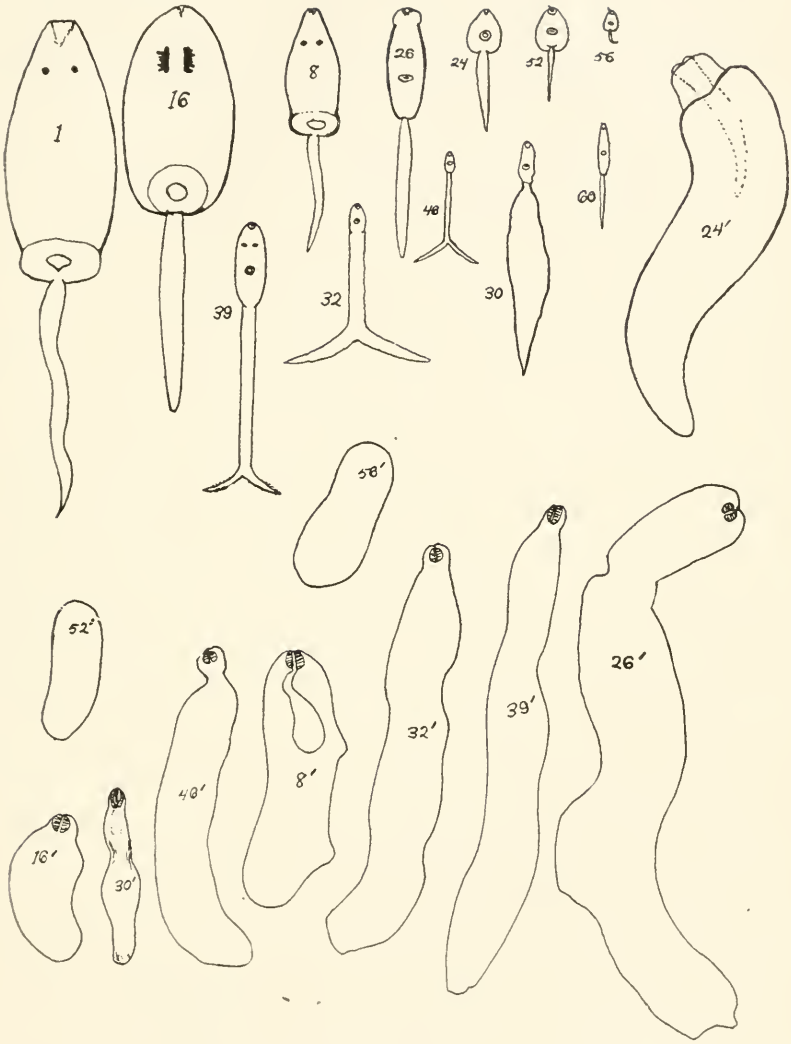
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THE

KANSAS UNIVERSITY SCIENCE BULLETIN.

VOL. X, No. 8—JANUARY, 1917.

(Whole Series, Vol. XX, No. 8.)

CONTENTS:

HISTOLOGY OF ASTRAGALUS MOLLISSIMUS. . . . . *Neva Ritter.*

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Histology of *Astragalus mollissimus*.

BY NEVA RITTER.

"LOCO" in Spanish may be translated "crazy." The stock on the range in the western part of America were, therefore, said to be "locoed" when they exhibited symptoms of a disease involving loss of muscular control.

The first published account of the loco-weed disease was in the Report of the Commissioner of Agriculture for October, 1873, by Doctor Vasey. He attributed it to Astragali in California.

From then on every year appeared some new article, more of which were descriptions of the loco weeds and their effects on the stock than reports of discoveries of the true cause of the disease.

Professor Sayre ('86), of the University of Kansas, published articles in the Transactions of the Kansas Academy of Science and in the Report of the State Board of Agriculture describing the loco weeds in Kansas and telling of their effects on stock. He reported two loco weeds here, *Astragalus* and *Oxytropis* (*Aragallus*). He suggested the theory that the hairs on the leaves may by irritation cause the disease. In 1888 he reported having used the extract on himself and getting no resulting loco symptoms. In 1890 he separated some crystals which he said were inorganic and were not the poisonous principle. In 1892 he reported traces of alkaloid and said that loco weeds are not the cause of the disease of the stock. In 1898 he thought a poison develops in the animal which does not exist as such in the plant. In 1904 he put forth three possibilities of the cause of the disease:

(1) Irritation by hairs.
(2) Disturbance to digestion by eating much loco weed in the early spring.
(3) A poison develops during digestion.

Kennedy ('88) found no alkaloid nor poisonous principle when he used infusions in boiling water, dried powder, and an organic

acid from the plant. He experimented on a dog and came to the conclusion that the loco disease is due to the indigestible character of the plant.

Dr. Mary G. Day ('89) made a chemical analysis of loco weed. She experimented on a cat and rabbits. Her conclusions were that there is a poison in loco weed which will cause illness and death, and may be experimentally given and the same results obtained in cats and rabbits; and that the poison must be weak, or if strong, present in a very small amount.

Professor Mayo ('93) gave a good account of the symptoms. He said there is no evidence of a narcotic; the effect is due to malnutrition and malassimilation.

Ruedi ('95) experimented on sheep. In his chemical study he separated "locoin," a base, which he considered harmless, and an acid which is harmful.

Crawford ('08) claimed that barium in loco weeds is the poisonous principle, and tried to support his theory by numerous experiments on sheep and rabbits.

Marsh ('09) told of experiments on feeding and attempts at cure. He said advanced cases are hopeless; they may recover if taken early and put on good feed away from loco weed. He added that injections of sodium cacodylate or strychnine, or the two together for cattle, or Fowler's solution for horses, have been known to help. In 1912 he tried antidotes suggested by Crawford with no success.

Alsberg and Black ('12) tried to substantiate Crawford's contentions for barium, but failed to do so.

The loco disease in Kansas is nearly all due to *Astragalus mollissimus*, the purple loco weed. *Oxytropis* or *Aragallus lamberti*, the white loco or rattle weed, is more plentiful farther west. Of these the stock eat the *Aragallus* more readily, but the *Astragalus* is much the more poisonous. Other species of *Astragalus* and *Aragallus* have been found to be poisonous, but these two only are widespread enough to be of great economic importance.

Astragalus mollissimus of Kansas prairies has a short stem with long pinnately compound leaves, all very hairy, so that they appear silvery. The leaflets vary in number from few to thirty-seven or more. The root goes down quite deep. The flowers are purple, arranged on a spike, blooming the last of May and the first of June. These plants are perennial and remain green all winter. They grow in clumps scattered over the pastures, and are not uniformly distributed. They have many enemies, those

observed last summer being larvæ in the stem, insect galls, fungous diseases, and leaf miners.

Some of the theories proposed to account for the loco disease in stock caused by these plants are:

(1) The weed absorbs the juices of the alimentary tract, causes it to dry up and exert pressure enough to kill the animals.
(2) Overfeeding on loco weeds.
(3) A fungous parasite on loco weeds, Claviceps.
(4) The indigestible character of the plant.
(5) Malnutrition and malassimilation.
(6) Starvation.
(7) Irritation by hairs.
(8) Disturbance to digestion.
(9) Poison developed in digestion.
(10) Not due to loco weeds, but accompanying them.
(11) Alkaloids.
(12) Some poisonous principle not yet isolated.
(13) Barium salts.

The effects of the disease on animals have been described as various. However all agree on a few, as here given by Marsh ('09).

Slow staggering gait.
Rough coat.
Staring look.
Emaciation.
Lack of muscular coördination.
Extreme nervousness, shown in shying, rearing, etc.

To which might be added:

Lack of sensitiveness.
Hallucination.

A horse walking under a telephone wire several feet above its head will lower its head to get under, or when stepping over a stick or a rut in the road will lift its feet very high. The coat of the animal becomes rough, the eyes have a glassy look; there may be, in cattle, a sac of serous fluid under the chin. After the habit of eating loco weeds is formed the animals will hunt for them, and reject other food in preference to them. When locoed they are very hard to manage, and it is almost impossible to back a locoed horse. One cow is reported, when shut up to keep her away from the loco weed after she had the habit, to have run against a stone wall so hard as to kill herself. Starting to walk across a pasture, affected animals keep going until they come against some obstruction. A horse will walk through a barb-wire fence, not

noticing the cuts at all. They finally forget to drink even, and slowly starve to death, hunting loco weeds all the time, and eating nothing else.

If taken from the loco weeds at first they may be cured by shutting them up and feeding, but after the habit has become firmly fixed they are incurable. Prophylaxis is to rid the pasture of loco weeds, but this is practical only in small fenced lots. If fed plenty, so they need no more, in the winter, the habit is not nearly so readily acquired. Loco weeds are usually first eaten in late winter or early spring when the grass is dried up and these plants are still green.

One suggested possibility is that an alkaloid is the cause of the toxicity. Some analyses have shown it, others not. Dean Sayre has found a trace of alkaloid, but as much or more in alfalfa. Miss Watson ('78) found an alkaloidal reaction in the root, also a resinous body. Power and Cambier ('91) found toxic alkaloids, together with volatile oil, acetic acid, resin, albuminoid, and globulin.

Crawford ('08), while working to find the cause of loco poisoning, discovered a trace of barium, which was verified by spectroscopic examination. He measured .01 per cent barium oxide in the ash, which indicated 1.56 milligrams of barium sulphate in 1 gram of ash. In some of the soils in that vicinity he found, by similar examination, no trace of barium, nor in the well water. His experimental results show that barium in the proper amounts, as well as loco weeds, will kill animals; so his conclusion is that barium in loco weeds causes their toxicity. He removed barium with sulphuric acid and found the solution harmless. His extract of the dried material was not always active, which fact he thinks means that there is something in the fresh plant which aids the solution of the barium. His theoretical antidote is sulphuric acid.

Working on this basis, Marsh ('12) experimented with three lots of cattle. One lot were under normal pasture conditions; one was given drinking water containing the amount of sulphuric acid calculated to neutralize the amount of barium eaten in loco weed; the other lot was given drinking water containing a like amount of magnesium sulphate. The same proportion of each lot became locoed, thus showing that neither sulphuric acid nor magnesium sulphate are antidotes, as they should be if barium is the cause of the toxicity, so he makes the statement that barium is not the poison.

A laboratory investigation by Alsberg and Black ('12) on the barium question shows that barium is not responsible for the toxicity of loco weeds. Their evidence is: Many plants which are harmless contain as much or more barium than loco weeds; there are enough other metals present to explain the action attributed to barium; extracts of alfalfa, which are prepared similarly to extracts of loco weed which are toxic to laboratory animals, are found to be toxic to some laboratory animals; the barium is present in "at most but minute traces."

ORIGINAL INVESTIGATION.

ALKALOID. The alkaloid test is a reddish-brown precipitate with bromine or iodine. When the bromine reagent is applied to sections of the petiole, leaflet or stem, and a control (soaked in alcoholic tartaric acid for two days, then in water one day), no difference can be seen in the sections. The same is true when potassium iodide-iodine is used as an indicator. Both of these tests were repeated many times on all parts of the freshly picked plant, indicating no alkaloids.

As a control to the test for alkaloids, sections of nux vomica seeds, calabar beans and belladonna root were soaked in the solvent for alkaloids. These and fresh sections were tested for alkaloids at the same time with potassium iodide-iodine. A very noticeable difference was discernible. The fresh sections show a deep orange-brown color; the alkaloid-free sections a light yellow color, if any. So if an alkaloid were present in my loco weed, a difference in color between fresh and control sections should have been detected; but as there was none, the conclusion reached is that there is no alkaloid present.

SUGAR. Sugar is present in a fairly large amount. When sections of the leaf or stem are boiled in Fehling's solution, at first there is no change, but after boiling longer the brick-red precipitate appears, indicating that cane sugar or a glucoside is probably present. This is indicated in all the cells except the bast fibers and water tubes. Loco weed gathered at 9:30 at night showed the presence of a quantity of sugar in the green parts on boiling with Fehling's solution; that gathered early in the morning not so much.

STARCH. Starch is present in great quantities in the stem, in the pith, medullary rays, pericycle and cortex. In the petiole the starch sheath contains it abundantly, but there is little in the pith.

In the leaflet the palisade cells are full of starch grains. These grains are definite in shape and about three microns in diameter. Potassium iodide-iodine and chloroiodide of zinc are the indicators used.

Material gathered early in the morning showed much starch in the stem, but less in the green parts; that gathered at night showed an abundance in the green parts.

Protein. When Millon's reagent is applied to sections they immediately begin to turn pink, which color deepens on standing or on heating. Protein is thus found to be present in the pith, medullary rays, pericycle, cortex, and especially much in the food-conducting tissues of the stem. In the petiole the same is true except that the pith contains very little. It is uniformly present in the leaflet. Potassium iodide-iodine confirms these indications. Material collected in the early morning and at night seems to contain the same amount.

Mucilage. Mucilage is present in the stem, as shown by methylene blue. Sections soaked in water still give the mucilage reaction, showing that it is not completely soluble in water, but might swell. A section of petiole was taken, mounted in alcohol, a distance measured, then water applied at one side of the cover glass. The cells immediately began to swell, the measured portion, sixty microns, swelling to seventy-five microns in a few minutes. The cells could be seen to round out. The mucilage was found to be more abundant near the edge of the section than in farther. The leaflet shows mucilage also. This occurrence of mucilage is interesting, as it is from species of *Astragalus* that the gum tragacanth of commerce is obtained.

Oil. Sudan III shows minute droplets of oil, in the pith cells especially. Sections soaked in ether overnight show less of this. Pieces of stem and leaf were steamed, the stem being collected on cover slips. These were first thoroughly cleaned with ether and handled with forceps. After staining with Sudan III no drops of oil are seen; therefore I conclude no volatile oils are present.

Tannin. Tannin is indicated by a purple color appearing when ferric chloride is added to sections containing it. No indication of it could be discovered in stem, petiole or leaflet. The test was repeated several times, always on fresh material.

Resin. Sections were soaked in a saturated aqueous solution of copper acetate, which turns resin green. Observations taken

occasionally for several months show no indications of the presence of resin.

OXIDASES. Gum guiac solution will not turn blue except when oxidases are present. It does not turn blue with any part of loco weed, meaning there are no oxidases present. When hydrogen peroxide is added a dark-red color appears, instead of the deeper blue, which is usual for peroxidases. This may mean a peroxidase is present together with some modifying substance.

GLUCOSIDES. Sections of loco weed treated in the following ways were boiled with Fehling's solution a few minutes, then observations taken:

(1) Fresh sections, no crystals.
(2) Treated with potassium hydroxide overnight at room temperature, cuprous oxide crystals present.
(3) Treated with hydrochloric acid overnight at room temperature, cuprous oxide crystals present.
(4) Treated with sulphuric acid overnight at room temperature, cuprous oxide crystals present.
(5) Treated with alcohol overnight at room temperature, cuprous oxide crystals present.

Untreated sections show crystals on long boiling with Fehling's solution. The same results were obtained when the treated sections were sealed with Fehling's solution and left in a warm place a few days. Sections of material preserved in formalin and treated in the same way give the same results.

These experiments may mean that glucosides are present which are broken by the various reagents used, giving the test by means of the freed sugar.

Crawford says that when sulphuric acid is added to an aqueous solution of harmful loco weed, it is rendered harmless because the barium is taken out of solution and made into barium sulphate. A section of loco weed was sealed with Fehling's solution and kept in a warm place. One week later crystals of cuprous oxide were noticed, showing that no glucose was present at first, or the crystals would have appeared sooner. It was probably a glucoside broken up by long standing and heating in Fehling's solution. Sections were soaked overnight in dilute sulphuric acid at room temperature. The next morning they were sealed with Fehling's solution. After five hours they contained many crystals of cuprous oxide. What happened in a week with Fehling's solution took place in a small fraction of that time with sulphuric acid. I think the change in toxicity in the solution which Crawford used was

due to the glucoside being broken up and thus being rendered harmless, instead of the barium being taken out of solution.

Crawford also states that in dried plants found by him to be inactive the barium has been converted into an insoluble form by drying. Sections of dried plants give a sugar test more readily than formalin or fresh material. This may be due to the glucoside being broken up in the process of drying. Crawford found aqueous extracts of the dried material ineffective, but experiments by others with feeding the dried material show it to be as toxic as fresh. This glucoside is soluble in water, as water in which sections have been soaked for some time show cuprous oxide crystals on heating with Fehling's solution, and the extracted sections have very few crystals in them.

From the experimental data at hand my conclusions are: That the toxicity of loco weeds may be due to some glucoside which is present, but which may be broken apart by various reagents; that the reactions which Crawford ascribed to barium are really due to this glucoside, the presence or absence of which can be proved more readily than the toxicity of such minute quantities of barium as he says are present in the plant.

ANATOMY.

THE LEAF. The leaf is compound, of few to thirty-seven or more leaflets. These as well as the petiole are densely covered by long unbranched hairs. (Figs. 1 and 2.) These occur equally on both sides of the leaf, about fifty per square millimeter. Their occurrence is shown in fig. 3. These hairs are composed of three cells, the basal cell, above this a very short cell with dense protoplasmic contents, and a very long terminal cell with thick cellulose walls. This terminal cell has no cutinized surface, as demonstrated by chloroiodide of zinc, Sudan III, or safranin-hæmatoxylin. There are small irregularities on the surface of this cell of the hair.

These terminal cells of the hair contain no living protoplasm. Methylene blue penetrates the cell wall, but does not color any contents. Hæmatoxylin stains the walls light violet, showing they are cellulose, but stains no contents, the center seeming clear and empty. Potassium iodide-iodine stains the contents of the middle cell deeply, but shows no contents whatsoever in the long terminal cell. Chloroiodide of zinc shows the middle cell stained dark yellow (the wall being cutinized and the contents protein),

but the long terminal cell wall only stains violet, while the cavity looks clear.

Loco weed leaves (dried) absorb 9.24 per cent of water, drops of water disappear when placed on them, and colored solutions (safranin, hæmatoxylin, eosin, and methylene blue) easily penetrate to the cell cavity. Haberlandt says that when such structures are present and the leaf is capable of absorbing much water (by weight) or drops of water when put on them, or there is quick penetration of colored solutions, "it is fairly safe to conclude that the hairs in question represent water-absorbing organs." Therefore I have concluded that these hairs function for the absorption of water in the form of dew or rain. The long cell gathers the moisture, the irregularities on its surface serving to keep the droplets from running off, then the middle cell with its contents conducts it towards the cells of the mesophyll. This middle cell has very dense contents, protein especially. The water may be taken along the terminal cell by imbibition, then drawn by osmosis through the middle cell into the leaf.

Cross sections of the leaflet show little difference in the upper and lower halves. The hairs and stomata (about thirty per square millimeter) are as numerous on one side as on the other. (Figs. 3, 4, and 5.) Palisade cells are arranged on either side of the central portion of the mesophyll, which contains the vascular tissues. It appears that the reflection of the sun from the ground is strong enough to stimulate the formation of palisade tissue on the under side of the leaf as well as to give light enough for the chloroplasts there to function. The terminal tracheids often have enlarged ends, which may serve for water storage. (Figs. 3 and 4.) A section of leaf seen through a microscope with a polarizer attachment shows only the cell walls of the hairs to look bright; nothing else can be seen in the dark field, and no crystals are in this way demonstrated in the leaf. The upper part of the basal cell and all of the outer cell walls of the epidermis are heavily cutinized. (Fig. 4.) A multiple epidermis is shown in a few places. The midvein of the leaflet consists of bast, phloëm and xylem.

The petiole has numerous bundles which supply the leaflets (fig. 6), one going out of the petiole into each petiolule.

There is a layer inside the walls of the bast fibers which stains reddish violet with chloroiodide of zinc, and yellow with potassium iodide-iodine. The lignified walls with both are yellow. With phloroglucin these linings stain red, but a little lighter than the walls. Methylene blue showed no mucilaginous modification.

These layers are therefore concluded to be inner layers of the bast which are not completely lignified, but still have cellulose in them.

With a polarizer, as well as potassium iodide-iodine or chloro-iodide of zinc, starch grains in the starch sheath are shown, but no crystals. Irregular broken spaces are often found in the center of the pith of the petiole. These do not seem to be mucilage channels.

Stem. The stem does not show distinct annular rings, even though secondary thickening is apparent. The phloëm is rather extensive (figs. 8, 9, and 10), and is filled with food materials. Fig. 9 shows three of the smaller bundles in a stem and fig. 10 a longitudinal section of one of the larger bundles. Sieve plates can not be seen. The older portion of the stem has thick cork. This cork tissue gives the suberin test with Sudan III and the lignin test with phloroglucin, as if the suberized membrane were partially infiltrated with lignin.

Root. The root shows annular rings better than the stem. Between the rings of water tubes are rings of wood parenchyma cells, which are unlignified, as are also the wood fibers. The water tubes are the only part of the root which is lignified. (Fig. 12.) The phloëm is great in extent. (Figs. 11 and 13.) The older portion of the root has cork similar to that of the stem.

Inflorescence. The peduncle is, like the petiole, made up of many bundles which are to supply the pedicels and bracts at their bases. As these are opposite, the peduncle contains four less bundles above their separation than below. There is relatively more bast in this portion of the plant than in any other. There are one or two rows of lignified parenchymatous cells, making a ring connecting the bundles, near the outer edge of the xylem.

The flower is a typical one of the tribe Galegeæ of the Leguminosæ. The pod is curved, with a partition extending in from the convex side and separating the two cells. In the center of the tissue of the pod is a layer .3 millimeter thick of bast fibers which encircle it, thus making it very stout.

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DESCRIPTION OF PLATES.

PLATE I.

FIG. 1. Hairs taken from a leaf. × 45.

FIG. 2. High-power drawing of hair from leaf, showing: *t*, terminal cell; *m*, middle cell; *b*, basal cell. × 450.

FIG. 3. Surface of a leaflet of loco weed, showing: *t*, tracheal system within the tissue; *e*, epidermal cells; *s*, stoma; *c*, cuticular ring at base of hair; *h*, hair. × 90.

FIG. 4. Cross section of a leaflet at the edge, showing: *e*, epidermis; *b*, basal cell of hair; *h*, hypoderm; *p*, palisade cells; *s*, stoma; *vb*, vascular bundle. × 115.

FIG. 5. Cross section of a leaflet at the midrib. × 115.

PLATE II.

FIG. 6. Diagram of a cross section of the petiole, showing: *e*, epidermis; *b*, bast tissue; *p*, phloëm; *x*, xylem. × 40.

FIG. 7. Cross section of a vascular bundle of the petiole, showing: *e*, epidermis, with *h*, hair; *c*, cortex; *s*, starch sheath; *t*, bast tissue; *p*, phloëm; *x*, xylem; *pi*, pith. × 90.

PLATE III.

FIG. 8. Diagram of a cross section of the stem, showing: *b*, bast tissue; *p*, phloëm; *x*, xylem. × 15.

FIG. 9. Cross section of vascular bundles of the stem, showing: *b*, bast tissue; *p*, phloëm; *x*, xylem; *mr*, medullary ray; *pi*, pith. × 90.

FIG. 10. Longitudinal section of a vascular bundle of the stem, showing the same tissues. × 90.

PLATE IV.

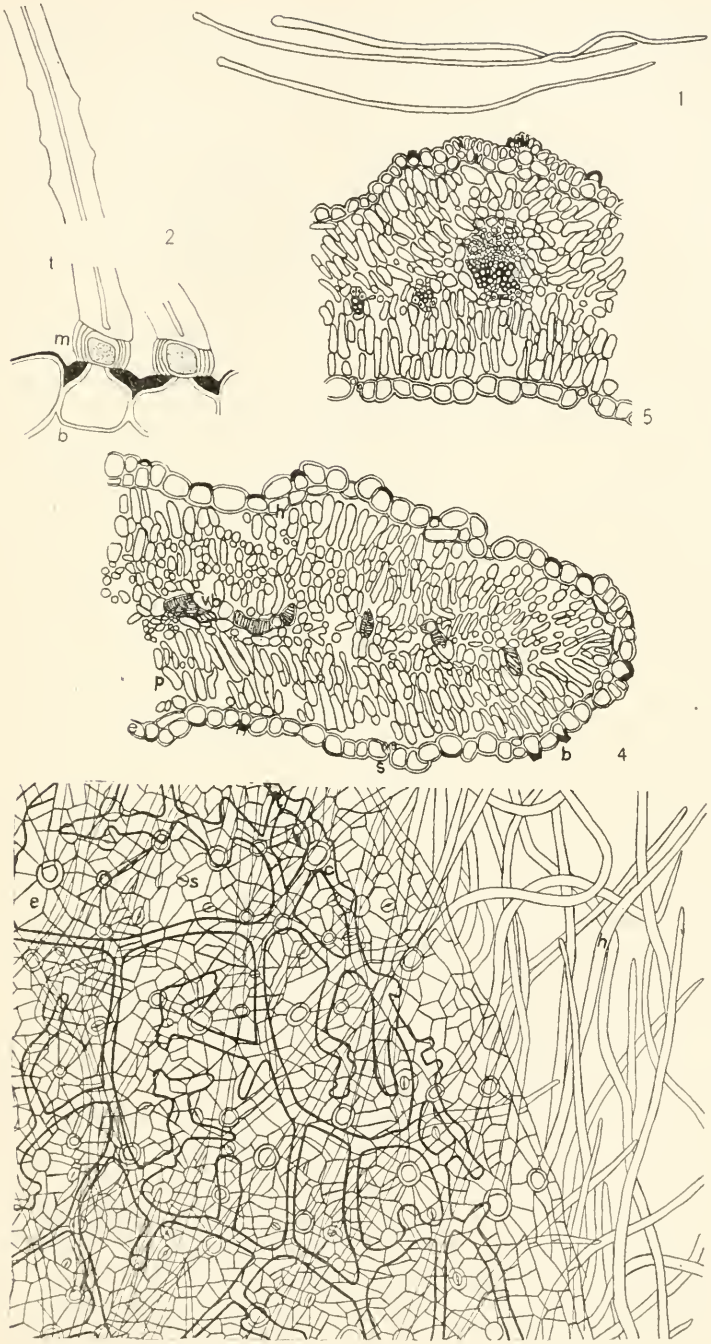
FIG. 11. Diagram of a cross section of the root, showing: *c*, cork; *p*, phloëm; *x*, xylem; *mr*, medullary ray. × 17.

FIG. 12. Cross section of a portion of the xylem of the root, showing: *mr*, medullary ray; *wt*, water tube; *wp*, wood parenchyma; *wf*, wood fibers. × 90.

FIG. 13. Cross section of a portion of the vascular bundle of the root, showing: *p*, phloëm; *mr*, medullary ray; *x*, xylem. × 90.

FIG. 14. Diagram of a cross section of the peduncle, showing: *e*, epidermis; *b*, bast tissue; *p*, phloëm; *x*, xylem. × 30.

FIG. 15. Cross section of a vascular bundle of the peduncle, showing the same tissues. × 90.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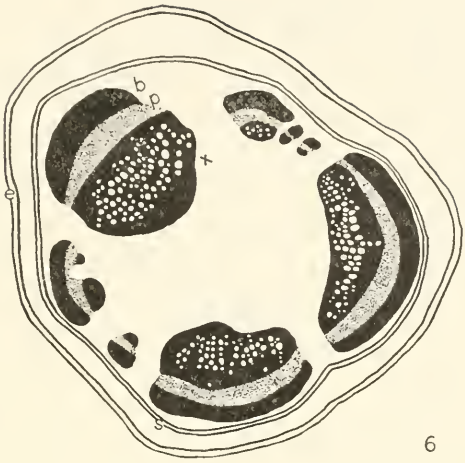
ASTRAGALUS MOLLISSIMUS.
Neva Ritter.

PLATE II.



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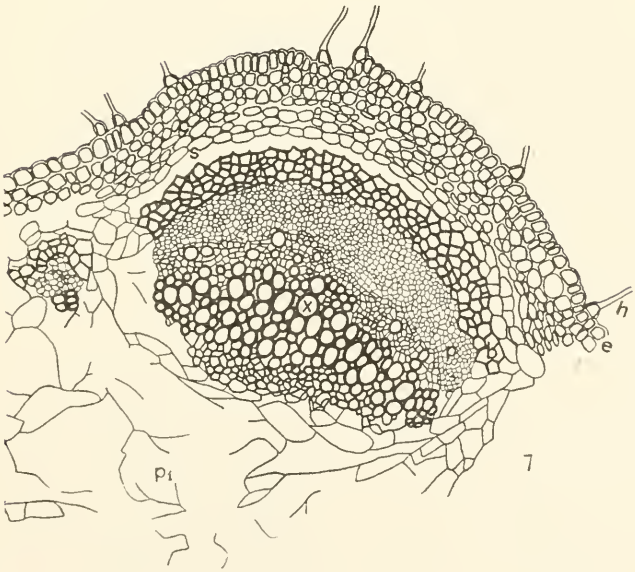

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ASTRAGALUS MOLLISSIMUS.
Neva Ritter.

PLATE III.

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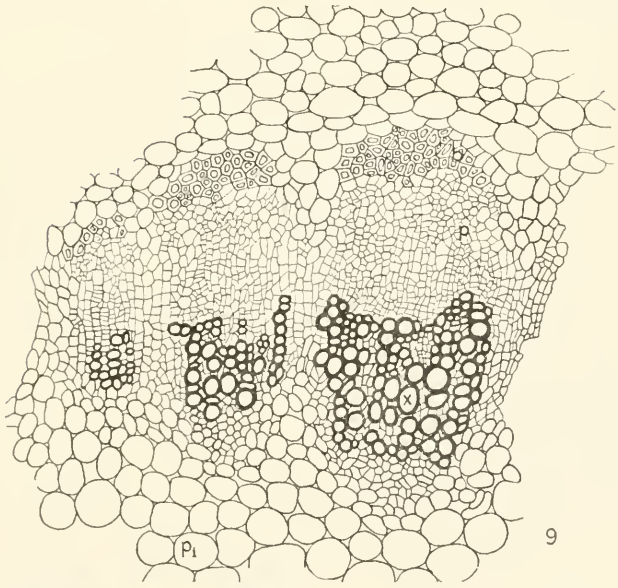

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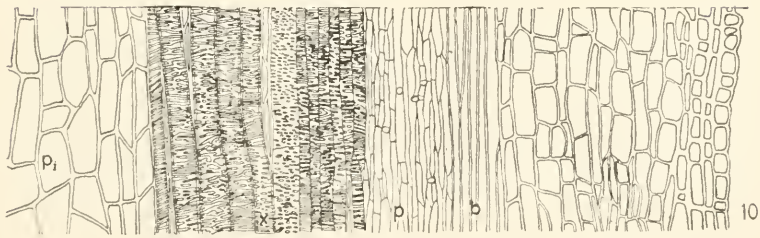

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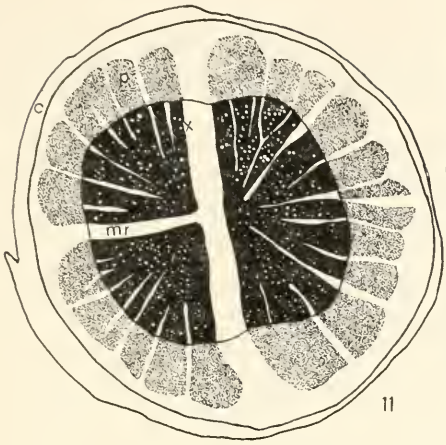
ASTRAGALUS MOLLISSIMUS.
Neva Ritter.

PLATE IV.



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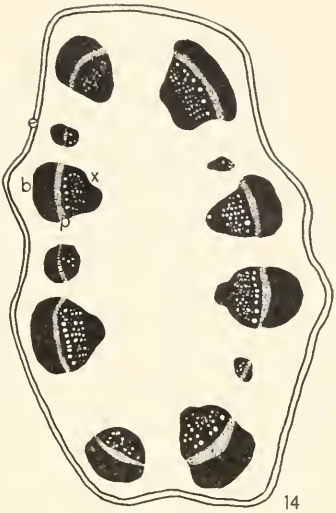

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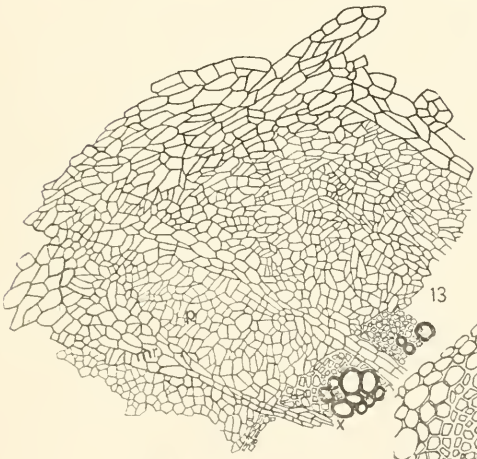

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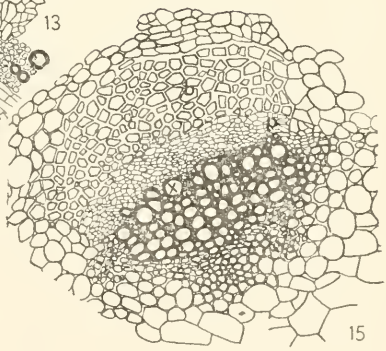

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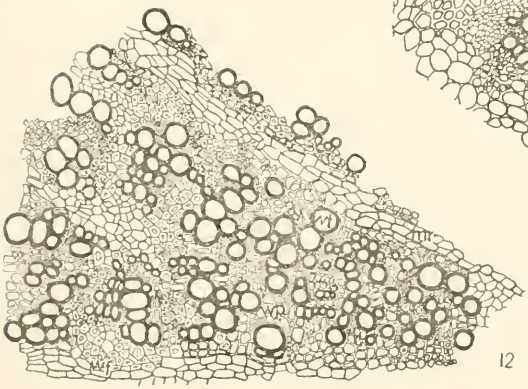

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THE

KANSAS UNIVERSITY SCIENCE BULLETIN.

VOL. X, NO. 9—JANUARY, 1917.

(Whole Series, Vol. XX, No. 9.)

CONTENTS:

ECOLOGICAL MORPHOLOGY OF ABUTILON THEOPHRASTI. . . *Louise Luckan.*

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VOL. X, NO. 9.] JANUARY, 1917. [WHOLE SERIES VOL. XX, NO. 9.

Ecological Morphology of *Abutilon theophrasti.*

BY LOUISE LUCKAN.

ABUTILON THEOPHRASTI, commonly known as velvet leaf, is an upright-growing mallow which may attain the height of six feet. It thrives at the edge of cultivated fields or in rich waste lands, such as lots, where it often occurs in dense patches.

During the summer of 1916 drought conditions prevailed for a month. The last rain of early summer fell about June 28 or 29, and the drought was not broken until the 26th of August. This meant that plant life would probably suffer for lack of water for at least a month. Many plants showed the effect of this lack of moisture very much, but *Abutilon theophrasti* remained fresh and green and attained its normal height. Here, then, an interesting problem offered itself as to what provisions the plant had made to withstand the drought.

The material for this study was collected on the south slope of the University campus. Both preserved and fresh material was studied. The standard histological methods were used and the drawings were made by the use of the camera lucida on the microscope and with the projectoscope.

ANATOMY.

The Leaf.

The leaves, borne on long, slender petioles, are roundish heart-shaped with an acuminate apex, and when full grown measure from four to five inches across. When the leaves are picked off they soon wither. During the hottest part of the day the edges of the leaf curl, but it requires only a short time for leaves to regain their turgor.

The shoots of young plants, each bearing from four to five leaves, were cut off, the cut surface sealed with wax and the shoots allowed to dry for an hour or more. These were then weighed separately and placed in a saturated atmosphere to see if they would regain their turgor or gain in weight. One set was left standing for forty hours, and then, after the leaf surfaces had been dried with filter paper, they were weighed. Those placed in the saturated atmosphere gained 15.11 per cent in weight, while those painted with distilled water and then placed in saturated atmosphere gained 18.95 per cent in weight. Another set was allowed to dry for three hours and was then brushed over with distilled water. The average of these tests showed a gain of 19.46 per cent in two hours. This shows that the plant has some power to absorb moisture from the atmosphere, especially if dew is present. Haberlandt ('14) gives two examples, *Convolvulus cneorum* and *Centaurea argentea*, where water is absorbed by hairs of the leaf. The wilted leaves gained 10 and 13 per cent, respectively, when immersed for twenty-four hours. In comparing the percentage gained and the conditions of the experiment, *Abutilon theophrasti* is much more efficient.

The leaf is dorsiventral, with the palisade tissue a single layer of cells on the upper side. Large thin-walled water-storage cells over the smaller veins interrupt this layer (fig. 1, *d*). Both surfaces of the leaf are thickly covered with four types of hairs, namely, two types of clothing hairs, one of which is stellate and the other an unbranched single-celled form, and two types of glandular hairs, one long and the other short. The stellate hairs, the most characteristic for the family, are the common clothing hairs, and these are found in great numbers and almost exclusively over the veins. The rays of the stellate hairs vary in number and length. They are united at their bases in the plane of the epidermis; and there being no stalk cells, these hairs do not rise far above the epidermal surface. The walls of the basal portion are relatively thick and contain pits (fig. 6). A ray cell (fig. 5) has the cavity diminishing toward the apex until in some cases it is entirely closed there. The walls of the rays show up as being composed of two layers—a thin outer cuticle imperfectly developed and a thick inner portion which the chloroiodide of zinc test gave evidence of being slightly infiltrated with cutin. The basal portion of these hairs stain a bright red with safranin, and yellow with chloroiodide of zinc, indicating cutinization, or possibly lignification; but give no color reaction with phloroglucin or ani-

line sulphate, and very little, if any, with Sudan III. In one test with the chloroiodide of zinc the inner portion of the cell wall of the basal region stained purple, indicating cellulose in the position shown in fig. 5. Some of these hairs showed nuclei situated in the basal part, but very little cytoplasm could be demonstrated.

Haberlandt, in his discussion of multicellular absorbing hairs, makes the statement: "If such a hairy covering is easily wetted and rapidly absorbs drops of water; if, further, the hairy leaf rapidly recovers its turgidity when immersed or besprinkled in the withered condition; if, finally, places for the entrance of water through the hairs are indicated by the presence of thin-walled basal cells with abundant protoplasmic contents, it may be safely assumed that the hairs in question serve to some extent for the absorption of water." The imperfect cutinization of the walls, above noted, would allow the water to pass to the inside of the hair. The structure of the stellate hairs, together with their occurrence only on the veins, and the amount of water absorbed by wilted leaves, as mentioned, lead me to believe that these hairs are water-absorbing hairs.

The other form of clothing hair is like a single ray of the stellate type; here the pits in the basal portion show up plainly (fig. 7).

The glandular hairs are of two kinds; one is long, being made up of from twelve to fifteen cells, while the other is only four to five cells in length. The basal cell of the long hair is enlarged (figs. 3 and 4). The cell just above it has its side walls cutinized throughout, so that it shows up as a bright-colored ring when stained with Sudan III or chloroiodide of zinc. The remaining cells of the hair are provided with a very thin cuticle. The apex is rounded out, and here drops of oil collect as it is secreted. The short glandular hair has its cells little differentiated, but the basal cell is slightly larger than the neighboring cells (fig. 8). These smaller hairs occur along the midrib and the larger veins, and Nestler ('99) says in some of the species of *Abutilon* they function as hydathodes, or hairs which excrete water. The hairs are advantageously situated for this, and as they contain living protoplasts they could possibly function in this way.

The stomata are not specialized, and are found in greater numbers on the lower epidermis, where they average 300 per square millimeter as compared with 250 on the upper surface.

The radial and outer walls of the epidermis are not especially thickened, but the inner wall of some of the epidermal cells becomes much thickened and mucilaginous and protrudes down into

the palisade tissue (fig. 2). The inner cell walls of the lower epidermis are not as thick as those of the upper, and only a few are mucilaginous. The mucilage may be so abundant, in varying degrees, that the inner wall at first glance might seem to be a second layer of epidermal cells, entirely filled with mucilage.

The mucilaginous deposits probably serve as a place of storage for water, absorbing a large amount when there is excess moisture in the leaf, and then gradually giving this up to the palisade tissue when the water supply runs low. I found no constant relation between these cells having the mucilaginous inner wall and the hairs, to indicate that water absorbed from the atmosphere by the hairs would be delivered to them for storage.

The midrib and the larger veins project prominently from the lower surface of the leaf, and the greater portion of this projection is composed of large thin-walled cells surrounding the vascular bundle (fig. 13). Kuntze ('91) describes mesophyll cells on the upper side of the vein, which break down to form large mucilage channels in many of the Malvaceæ. In the cross sections of the veins examined no channels were found, and only a single cell situated in this position showed mucilage (fig. 13, *m*). There are, however, on the upper surface a large number of epidermal cells, outlining the veins, which have a mucilaginous content (fig. 12). Therefore we may conclude that mesophyll cells of the midrib, as well as the mucilage cells, are good places for water storage, and are probably used as such.

The Stem.

The stem structure follows closely the type form of the dicotyledonous stem. The young stem is covered with both types of clothing and glandular hairs described, under the leaf. These hairs extend perpendicular to the surface, so that the young stem looks quite fuzzy. The epidermis of the stem has regular cells, the walls of which are not thickened to any great extent. The cuticle on the outer wall is very thin, and I found no cutinized layer which would aid in keeping in the water.

The cortex is made up of four regions—an outer thin-walled parenchyma, a collenchyma, a second thin-walled parenchyma, and the starch sheath (fig. 15). The outer thin-walled parenchyma is made up of three layers of radially enlongated cells. The collenchyma tissue is composed of three layers, forming a broken ring, with the gaps opposite the medullary rays. This arrangement allows the water to pass in and out to the epidermis more

readily than if it were to diffuse through the thickened walls of the collenchyma. The second layer of thin-walled parenchyma of the cortex is only one or two cells deep and is often crushed up against the collenchyma. The starch sheath is apparent, but the cells do not contain starch.

The bast strands of the pericycle are in groups, separated by thin-walled cells. The cell walls of the bast are lignified. Besides the bast of the pericycle there are several layers of secondary bast strands formed by the cambium and interspersed in alternate layers with the secondary leptome elements (figs. 14 and 15).

This growth develops a wedge tapering outward, so that in cross section the stem has wedges of bast and phloëm alternating with wedges of medullary ray, which taper in the opposite direction (fig. 14).

The medullary rays are only one to two cells wide in the xylem region of the stem, but where they extend out through the phloëm portion of the vascular bundle they broaden radially, so that here they are from four to six cells wide (fig. 15). The medullary rays of the stem do not contain large amounts of starch.

The phloëm is conspicuous, for there are between eight and ten layers of cells between the inner bast strands and the cambium.

Although this plant is an annual, the xylem of the stem seems to be made up of rings of growth due to alternate layers of tracheal tubes and xylem parenchyma (fig. 15).

The pith is permanent in the stem, increasing with the other tissues, so that in mature stems the pith has a diameter of one centimeter. In the younger pith the outer cells are filled with starch grains, and among these cells filled with starch are mucilage channels, or sometimes mucilage cells (fig. 14). These channels are formed by the cell walls becoming mucilaginous and some of them breaking down. They may be made of only one cell in the cross section, or several cells adjoining may break down so that the channels or pockets are of different sizes. The pith cells contain many calcium oxalate crystals, usually compound, which are very noticeable in the young cells, where they fill the cell cavity.

The Root.

The root is a long taproot with few secondary roots. The root resembles the stem in having large wedges of bast. The medullary rays, especially in the region of the xylem, are well filled with starch. Cork is present as an outer covering of the older portions of the root (fig. 16).

In the thin-walled parenchyma of the cortex, droplets of oil are of frequent occurrence, and these were discovered to be held in the stroma of spherical elaioplasts, which in unstained sections appear simply as highly refractive bodies. When treated with chloroform or ether for twenty-four hours and then tested with stains for oil, no oil was demonstrated, but irrigation on a slide with solvents of oil for ten to fifteen minutes did not cause all of the oil to disappear. Sections from celloidin material, which had been fixed with one per cent chromacetic acid, tested with Sudan III, alkanin and chloroiodide of zinc, showed these bodies as globular masses reacting as oil. Strasburger ('13) makes the statement that the chromacetic acid fixitive causes percipitation of the stroma of elaioplasts and renders the oil less soluble. This may explain oil remaining in the stroma of the elaioplasts through the celloidin process in which solvents of oil are employed. Safranin-hæmatoxylin stain and the three-color stain showed these bodies as purplish, spherical, reticulate structures. The elaioplasts in the cells of the root sometimes lie next to the nucleus, but a constant relation was not found. The cytoplasm in the older cells of the cortex is only a thin layer lining the wall, so that there the elaioplasts are against the wall; usually only one to a cell (figs. 17 and 18).

Beer ('09) in his work on *Gaillardia* sustains the theory of Wakker ('88) that the elaioplasts are formed by the aggregation and fusion of the leuocoplasts in the cell. His work seems to give conclusive evidence for this mode of formation in the plant studied. He found elaioplasts in different stages of development, and observed living cells in which the process was going on where cell divisions had long since ceased; but Politis ('11), in his work on the development of the elaioplasts in *Ornithogallum umbellatum* bulbs, maintains that they are formed by the cytoplasm, almost simultaneously with nuclear division. He states that the stroma of the elaioplasts stains with the nuclear stains, reacting like the nucleolus; and since they also are dissolved with nuclear solvents, he maintains the elaioplasts are of nuclear origin and gives the fact of the close association with the nucleus and the chemical reactions as basis for this conclusion.

In *Abutilon* I found elaioplasts were present in the actively growing parts, such as floral organs, growing apices of stem, young seedlings, barely sprouted seeds, and also in root sections in all stages of development. When the older parts of the stem were tested no elaioplasts could be demonstrated. The presence

of elaioplasts in the growing apex of the stem and the absence of them in the older stem seems to support Politis' theory that they are active only a short time and are then used up by the plant tissues, but the fact that the elaioplasts are present in the cells of the cortex of the old root is against this.

In regard to their origin, I am not able positively to confirm either theory. I find leucoplasts aggregated about the nucleus in the cells of the soaked seed, but I could not find intermediate steps, for it seems that in my material the elaioplasts do not form oil until they have attained a spherical form and ultimate size. Certainly the hairs of the *Gaillardia* studied by Beer are unusually favorable subjects for showing the course of elaioplast development.

Politis found the elaioplasts usually present in the epidermis of the floral organs, but their occurrence in other organs was inconstant. He says they are an active organ of the cell during growth. When active the elaioplasts have a rotating motion in the cell; when at rest the elaioplasts may assume various forms as a last phase of development. He found elaioplasts present in the following species of Malvaceæ: *Hibiscus syraicus, Althea rosea, Malva rotundifolia, M. sylvestris, Gossypium arboreum, Gothea cauliflora.*

MICROCHEMISTRY OF CELL CONTENTS.

The Leaf.

Leaves that had been in bright sunlight, brought in and tested for starch with iodine, showed the presence of starch. Similar leaves boiled in Fehling's solution showed crystals of cuprous oxide, indicating the presence of sugar in the form of monosaccharide.

Sections of leaves in Sudan III overnight showed palisade cells orange yellow, but not good demonstration of oil. The large glandular hairs showed droplets of bright orange red, indicating oil. Leaf sections soaked in chloroform and then tested with Sudan III did not give the red color reaction.

Volatile oil tests were made by collecting the distillate from leaf tissues on cover slips rinsed in chloroform, which were then treated with Sudan III, and iodine fumes. On cover slips without the distillate, but otherwise treated in the same way, no oil was demonstrated; on those with the distillate, however, a good positive test was shown, indicating the presence of volatile oil in the leaf.

Sections of leaves were treated with methylene blue as a mucilage test. The inner cell wall of some of the epidermal cells took the stain (figs. 1, 2*b*).

Sections of the leaf heated in Millon's reagent showed brick-red contents in the palisade and spongy parenchyma cells.

Sections treated with fresh ferric chloride solution gave no tannin reaction.

Compound crystals of calcium oxalate were demonstrated by the use of hydrochloric acid, in which the crystals disappeared without effervescence. These crystals were common in the border parenchyma of the large veins.

Stem.

Sections of older stems treated with iodine showed the presence of much starch in the outer cells of the pith and also in the medullary rays.

Fehling's solution used on sections of the stem showed cuprous oxide crystals, and these increased in number and size when sections were left in a incubator for several hours, thus indicating the possible presence of saccharose and glucosides in addition to glucose.

Young stems showed the presence of oil in elaioplasts, but in older portions of the stem the sections gave no oil reactions. The glandular hairs of the stem and the petiole have drops of oil collected at their apices. Sudan III stains these drops, collected on a slide, a bright red. When left on a slide on a 50° oven for twenty-four hours the oil did not disappear. It is thus probably fatty oil.

Protein tests were made with Millon's reagent, chloroiodide of zinc, and iodine, showing protein present only in the region of the phloëm and cambium. Methylene blue showed mucilage present in the pith and a very little in the cells of the cortex, and when fresh sections of the stem were placed on the slide and water applied to them, swelling and finally solution occurred in the secondary thickenings of the walls of some of the pith cells.

Calcium oxalate crystals are present in cells of the cortex and young pith.

Sections treated with ferric chloride gave no evidence of tannin.

Sections treated with saturated copper acetate solution for three weeks failed to show the presence of resin.

Alkaloid tests were made with potassium iodide and chloroiodide of zinc. Other sections soaked in alcoholic tartaric acid, a solvent of alkaloids, were compared with fresh sections. No brown precipitate was demonstrated, leading to the inference that no alkaloids were present.

Root.

Starch is present in the medullary rays. Methylene blue showed very little mucilage in the root. It was present only as a thin film in a few of the cell walls of the cortex. Fatty oil is present in the stroma of elaioplasts. Fresh sections soaked in solvents of oil, choloroform, ether and xylene, and then stained with Sudan III, showed the oil had been dissolved out and the stroma was partially collapsed. Tests for volatile oil were negative.

Protein tests are not decisive, because the cell walls of the lignified tissue became brick red with Millon's reagent. When these walls were tested with aniline sulphate and phloroglucin, they gave the lignin reaction.

Seed.

Sections of soaked seed treated with Sudan III and iodine showed a large amount of oil in the endosperm and also in the embryo.

Sections of seed treated with Millon's reagent turned red in the region of the endosperm and cotyledons, indicating the presence of protein as a reserve food. Other sections treated with iodine showed no starch was present.

Methylene blue showed no indications of mucilage.

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DESCRIPTION OF PLATES.

PLATE I.

FIG. 1. Section of leaf showing part of a large vein. × 300. *a*, upper epidermal cell; *b*, mucilaginous wall; *c*, palisade cell; *d*, water-storage cell; *e*, spongy parenchyma; *v*, vein; *f*, stellate hair; *g*, glandular hair.

FIG. 2. Cross section of leaf. × 285. *s*, stoma. The rest of the index is the same as in fig. 1.

FIG. 3. Long glandular hair. × 200. *a*, apex, *b*, basal cell; *c*, protoplast shrunken away from wall.

FIG. 4. Lower portion of long glandular hair from section of the petiole. × 325. *b*, basal cell; *d*, cutinized wall; *e*, cuticle.

FIG. 5. Longitudinal section through stellate hair, showing peculiar reaction in chloroidide of zinc; stipled, orange yellow; shaded, purple. × 325.

FIG. 6. Cross section of leaf showing stellate hair over vein; index same as fig. 1. × 325.

FIG. 7. Simple clothing hair from section of petiole. × 325.

FIG. 8. Short glandular hair. × 325. *a*, plasmolysed protoplast; *b*, basal cell.

PLATE II.

FIG. 9. Surface view of leaf, showing relation of hairs to the veins. × 200. *a*, long glandular hair; *b*, short glandular hair; *c*, stellate hair; *d*, stoma; *e*, vein.

FIG. 10. Tangential section of leaf. × 200. *a*, palisade cells; *b*, border parenchyma; *c*, tracheal tube.

FIG. 11. Tangential section of leaf, showing frequency of the mucilage cells. × 200. *r*, stoma; *h*, hair; *m*, mucilage.

FIG. 12. Tangential section of leaf, above a vein, showing frequency of mucilage cells there. × 200.

FIG. 13. Cross section of large vein. × 100. *a*, thin-walled cells; *p*, phloëm; *x*, xylem; *m*, mucilage.

PLATE III.

FIG. 14. Cross section of stem, showing the regions and the wedged effect of bast and medullary rays. × 15. *c*, cortex; *b*, bast; *p*, phloëm; *x*, xylem; *m*, mucilage; *mr*, medullary ray; *s*, starch in pith.

FIG. 15. Section of stem, showing rings in xylem. × 150. *a*, epidermis; *b*, thin-walled parenchyma; *c*, collenchyma; *d*, second thin-walled collenchyma; *e*, starch sheath; *f*, bast fiber; *g*, leptome elements; *h*, phloëm; *i*, cambium; *j*, water tubes; *k*, wood parenchyma; *l*, pith; *m*, mucilage channels; *n*, medullary ray.

FIG. 16. Cross section of root. × 15. *c*, cork; *b*, bast; *p*, phloëm; *x*, xylem; *mr*, medullary ray; *r*, rootlets leaving the root.

FIG. 17. Cells of cortex of seedling root. × 325. *e*, elaioplasts.

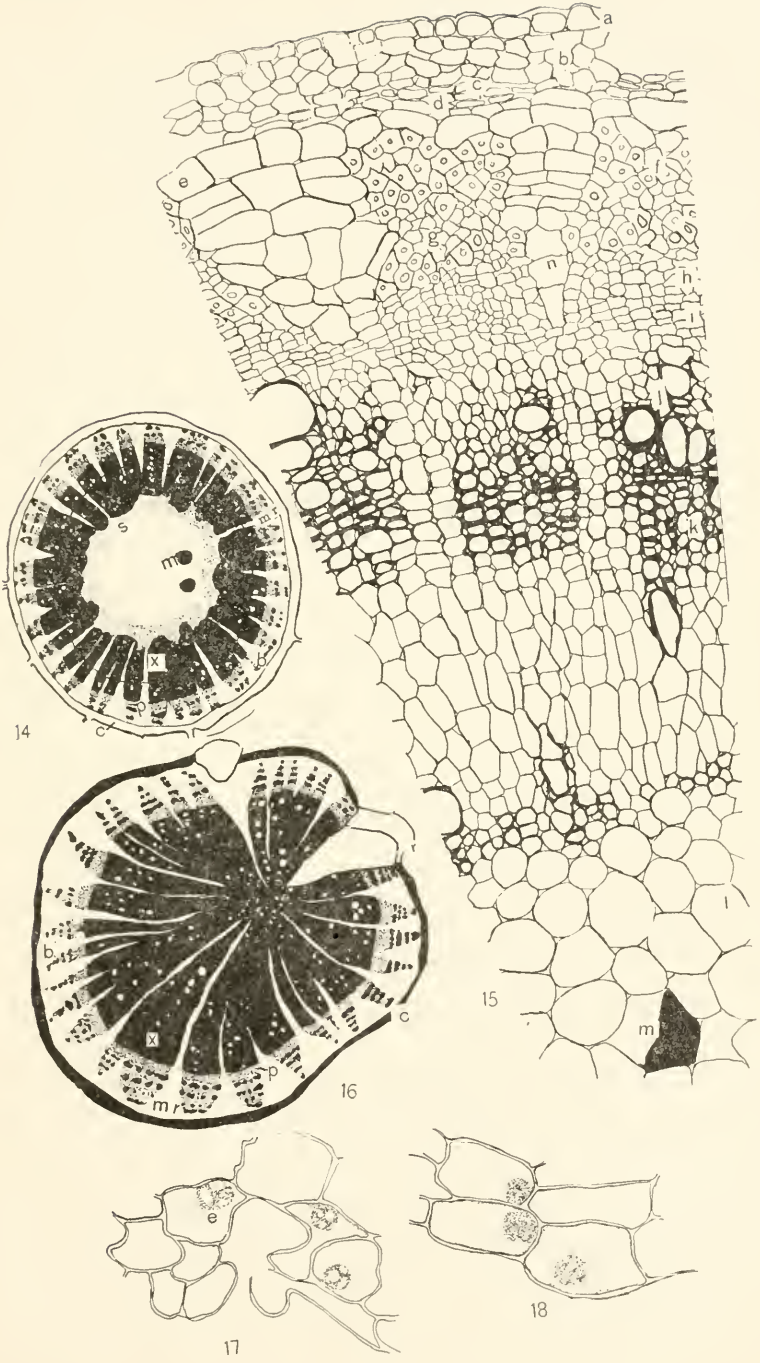
FIG. 18. Cells of cortex of older root. × 325. Showing elaioplasts.

ABUTILON THEOPHRASTI.
Louise Luckan.

PLATE I.

ABUTILON THEOPHRASTI.
Louise Luckan.

PLATE II.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THE

KANSAS UNIVERSITY SCIENCE BULLETIN.

VOL. X, No. 10—JANUARY, 1917.

(Whole Series, Vol. XX, No. 10.)

CONTENTS:

ERYOPS; ERYOPSOIDES, GEN. NOV. FROM THE NEW MEXICO PERMIAN.
Herman Douthitt.

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VOL. X, NO. 10.] JANUARY, 1917. [WHOLE SERIES VOL. XX, NO. 10.

Eryops; Eryopsoides, Gen. Nov. from the New Mexico Permian.

BY HERMAN DOUTHITT.

SKULL STRUCTURE.

THE statements made herein are based upon the study of eight skulls in the University of Chicago collection, some incomplete, and some only partly uncovered at the present time; one incomplete skull loaned from the Yale Museum for the purpose (No. 826); one skull and fragmentary material from the University of Kansas collection; and descriptions by Branson, Case and Broom. Shortly after this work was undertaken, in the fall of 1913, a paper by Broom appeared which covered to a large extent the same field. The morphological part, therefore, is presented mainly as a review and criticism of the work of Broom.

The structure of the upper surface of the skull was found to offer no disagreements from the results made known by the investigations of Branson, Case and Broom, and need not be discussed here. A few slight disagreements were found, which represented probably mere individual differences.

The occipital region and base of the skull were found to agree with the accounts of Broom, except that there is considerable evidence that the supraoccipital is present. No separation could be made out of the elements designated by Broom as basisphenoid and sphenethmoid, but there appears no reason for doubting his determination. As regards the supraoccipital, evidence of its presence is provided by one small, apparently immature, skull. The sutures extend from just dorsad of the condyles, dorsad and laterad, the supraoccipital thus forming more than one-half of the border of the foramen magnum. None of the larger skulls at hand give satisfactory evidence of its presence, though several are well preserved.

It seems, therefore, that the supraoccipital was present in *Eryops*, but that in adult life the element was fused with the exoccipitals. Its presence would be logically expected, since the postparietal is clearly excluded from the foramen magnum. To have the two exoccipitals meet and fuse above would be decidedly unusual; and even if no trace of sutures were to be found, it would be logical to interpret the upper part as supraoccipital and the lateral as exoccipital. The old assumption that the supraoccipital is absent in the Amphibia can not be given any consideration, for the writer has made it out in *Trematops*, and it will probably be found in other Temnospondyli when looked for.

Von Huene (1912) supposed the supraoccipital to be present, and showed it in his drawings of the occiput. The part he designated as supraoccipital, however, forms less than one-third of the element.

The palates are poorly preserved in all of the Chicago specimens, from which the matrix had been removed, but as far as their elements could be made out, they agree with the descriptions of Case and Broom. As regards the dentition of the palatine, transverse and vomer bones, however, there is difference of opinion. Branson and Broom concluded that each of these bear one large tooth at a time, and explain the cases of two occurring at once as being due to the development of a second tooth, to replace the first, before the first happens to be shed. Case believed that there were normally two on each bone, with which view the writer is agreed. The number of cases in which two teeth are present and indistinguishable as to size or appearance in the specimens at hand, and in published drawings available, seems to show that two is the normal number, and that a less number is due to one or both being lost in preservation. The following table shows the conditions found:

Element.	Number examined.	Number having two teeth.	Number having none.	Number having one.
Vomer	16	5	9	2
Palatine	15	6	5	4
Transverse	13	4	6	3

It will be seen that two teeth are preserved about as often as one. These two, it may be mentioned, are similar in size and appearance in nearly every case. The fact that there are so often no teeth at all suggests strongly that these were broken off and lost, either before or after death, but more probably afterwards, and that where only one tooth is present the other has been thus lost. Owing to the large size and prominence of the teeth, a slight

amount of shifting about of the skull after death must have resulted in their being broken off, and indeed they must have been often broken off in life. The presence of fresh scars indicates the same fact. Where several scars are present, the extra ones will present a different appearance, owing to the healing of the broken surfaces.

Broom speaks of the surangular as an element of the lower jaw, and leads us to suppose that it is present as a separate element. Were this true it would be the only case known of an amphibian with a separate surangular bone. A careful examination, however, of several good jaws of the Chicago collection shows not the slightest evidence of the separation from the articular of the part of the jaw so designated. That this portion of the articular of the Amphibia was once a separate element in their ancestors, and corresponds to the surangular of reptiles, seems probable; but the fusion in *Eryops* is as complete as in other Amphibia. A well-preserved jaw of the Chicago collection shows the three coronoid bones as described by Broom.

A COMPARISON OF TEXAS AND NEW MEXICO MATERIAL.

The vertebrate faunæ of the Texas and New Mexico Permian are widely different from each other. Aside from the genus *Eryops*, only two genera, *Edaphosaurus* and *Diadectes*, are recognized to occur in both. *Dimetrodon* and *Clepsydrops* have been reported from the New Mexico Permian, years ago, but the negative evidence of later, more careful investigations is against their presence. Moreover, it is not unlikely that more complete specimens will show that those remains that have been referred to *Diadectes* represent really a closely allied but distinct genus. With such slight similarity between the faunæ of the two regions, there is especial need to make careful comparative studies of the specimens from the two regions that have been referred to these genera, in order to determine whether they are really generically identical.

Material and information concerning the New Mexican representatives of *Eryops* are scarce. Marsh (1878) first reported *Eryops* from New Mexico, but supposed he was dealing with the remains of a reptile, and described them under the name of *Ophiacodon grandis*. He gave us no information of value as to structure. Cope, in 1881, gave us the following description of *Eryops reticulatus*, without figures:

"The most prominent peculiarity of this species is seen in the neural spines, which are not expanded at the summit, as in *E. megacephalus*, but have rather contracted apices, Another character is the sharply reticulate

sculpture of the maxillary bones. The species is much smaller than *E. megacephalus*, or even than *T. insignis*, and the extent of the ossification of the vertebral elements is intermediate between the two species. The inferior surfaces of the intercentra are smooth, and the diapophuses are compressed. The occipital condyles are depressed, and not very well distinguished inferiorly. The humeri have expanded extremities, with enlarged epicondyles, well-developed condyles, and no epitrochlear foramen. Width of occipital condyles, m. .016; elevation of dorsal vertebræ, .024; width of intercentrum, .011; length of intercentrum (below), .207; five maxillary teeth in .015."

According to Case, the material upon which the description of Cope was based was mingled with the remains of other animals. Of Cope's material only the intercentra are known to-day, according to Case. We can not be certain, therefore, that the spines described by Cope did not belong to some other animal. Williston (1911) described briefly Marsh's material, but made no anatomical studies.

The present comparative studies are based upon two incomplete and poorly preserved skulls from New Mexico, one from the Yale Museum (No. 826), and one from the collection of the University of Chicago; and ten skulls from Texas, in the museum of the universities of Chicago and Kansas, and the published drawings of Case and Broom for specimens in the American Museum. Unfortunately, the skulls from New Mexico are in such poor condition that no satisfactory measurements or determinations of sutures could be made. The skulls are apparently shorter and broader than those from Texas, but this can not be established. There is one constant difference which is discernible, however, which is, in the opinion of the author, of generic rank. This is in the matter of the arrangement of teeth on the palatine, transverse and vomer bones.

In the skulls from New Mexico the two large teeth on each of these elements are without exception transverse in arrangement, while in the skulls from Texas they are without exception longitudinal with respect to each other, where both are present, or where fresh scars are present. Specifically, the condition in the skulls from New Mexico is as follows: Two transverse bones, one with two teeth arranged transversely, one with one tooth; three palatine bones, two with two teeth arranged transversely, and one with one tooth; four vomers, two with two teeth arranged transversely, one with one tooth and a fresh scar, arranged transversely, and the other with one tooth. In all the Texas skulls at hand there is not a single exception to the rule that the two teeth on each of these elements are longitudinal with respect to each

other, either where two teeth are present or where there are fresh scars.

Considering that there is not an exception in all the skulls or published drawings available, this character seems to indicate that the New Mexican specimens are distinct from those from Texas. No doubt, better-preserved material will show other important differences. If the materials described by Cope be of this animal, then the New Mexican specimens have the apices of the neural spines contracted, and differ in the character of the intercentra. The skulls at hand from New Mexico seem to be shorter and broader than those from Texas, but owing to the imperfection of the material this can not be proved. But until better material is secured we must rely upon this one character, which should be of generic rank. For the new genus the name *Eryopsoides* is proposed, and specimen No. 826 of the Peabody Museum is named as type. Whether Marsh's *Ophiacodon grandis* (1878) and Cope's *Eryops reticulatus* (1881) are the same species will perhaps never be known, since the type materials are lost; but since Marsh's name precedes, it should be adopted as the specific name for the New Mexico specimens referred to *Eryopsoides*.

While there is no particular interest in the mere fact of recognizing a closely allied but distinct genus, the recognition is in this case of considerable interest, since it shows that *Eryops* is not common to both regions. The faunæ of these regions, so far as known, have very little in common, *Edaphosaurus* and *Diadectes* being now the only forms recognized as common to both. It is not at all improbable that better-preserved specimens will show that the New Mexico specimens referred to *Diadectes* are really generically distinct, and that *Edaphosaurus* is the only genus common to both regions. This would indicate a faunal separation of the two regions, and the nature of the animals is such as to show that they developed parallel, rather than that they were separated in time, and one descended from the other.

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A Study of Several Strains of Pleomorphic Streptococci.

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From the Department of Bacteriology of the University of Kansas, Lawrence.

KLEIN AND GORDON[1] isolated a polymorphic streptococcus from a series of scarlet fever cases and called it *Streptococcus scarlatina*. They considered it identical with the *Streptococcus conglomeratus* of Kurth.[2] Klein held that this streptococcus is causally related to scarlet fever in man and is wholly distinct from *Streptococcus pyogenes*. Gordon believed that both *Streptococcus pyogenes* and *Streptococcus conglomeratus* may play a part in the causation of scarlet fever, but that *Streptococcus conglomeratus* is the more important of the two, and that it occupies a position in the bacteriological kingdom between *Streptococcus pyogenes* and *Bacillus diphtheria*.

Winslow[3] mentions the fact that cocci isolated freshly from the mouth are particularly apt to show successive pairs of flattened cells and occasionally exhibit elongated rod-like cells, and that large cells sometimes appear in chains of smaller cells. This is probably a generally observed fact, although practically no mention is made of it in the literature.

Newman[4] emphasizes an observation of Gordon that frequently the streptococcus may morphologically resemble the Klebs-Loeffler bacillus.

Jean Broadhurst[5] has more recently observed large cells appearing in sugar-broth cultures of streptococci, and remarks upon the absence of reference to such variation in the literature.

Behring[6] investigated the *Streptococcus longus* and recognized four groups. Under group three he places Kurth's *Streptococcus conglomeratus* as one yielding or producing scaly flocks of sediment. Andrew and Horder[7] have classified as *Streptococcus anginosus* a pathogenic long-chained form, allied in other respects to *Streptococcus salivarius*, and bearing to it much the same relation which

Streptococcus pyogenes bears to *Streptococcus mitis*. They mention it as occurring in cases of scarlatinal and other forms of sore throat. It produces a flocculent deposit in broth, clots milk, reduces neutral red, forms acid in saccharose, lactose, and raffinose.

The enterococcus of Thiercelin, Escherich, Beeson[8] and others is a pleomorphic streptococcus that varies from the streptococcus of Gordon in its growth in broth, milk and on agar.

The object of this paper is to report upon a pleomorphic strain of the streptococcus isolated from chronically enlarged cervical lymph glands of a patient otherwise apparently in good health, and to compare this with eight other more or less markedly pleomorphic streptococci isolated from five cases of tonsilitis, two cases of bronchitis, and one case of infection on back of hand and with *Streptococcus pyogenes* and *Bacillus diphtheria*. The basis of comparison is upon morphology, staining reactions, cultural characteristics, action on carbohydrates according to Andrew's and Horder's classification, and complement fixation tests.

In November, 1912, a local physician requested a bacteriological examination of material from the cervical lymph glands of a patient with the following history: Male, age 26; had noticed twenty-six months previously the enlargement of the cervical lymph glands on the right side of the neck. Nineteen months later he attended a hospital clinic, drainage was established, and the case observed at intervals by the attending physician for seven months. During this time the glands continued to enlarge. At the end of seven months the patient went to his family physician, who suggested the bacteriological examination. No acid-fast organisms were found, but a pure culture of a marked pleomorphic streptococcus was obtained both from the glands and from the tonsils. At this time the glands on both sides were involved; the axillary and inguinal lymph glands were shotty. The physician advised a tonsillectomy, but the patient wanted a vaccine. Accordingly a vaccine was prepared. Three weeks after the administration of the first dose the swelling had disappeared and the patient was discharged. Three months afterward there seemed to be the beginning of a recurrence, a tonsillectomy was performed, and the patient has remained well to date.

The organism isolated had the following characteristics, which, in so far as morphology, staining reactions and general cultural characteristics are concerned, seemed to be identical with those of *Streptococcus scarlatina* of Klein and Gordon and the *Streptococcus*

conglomeratus of Kurth. It apparently varied somewhat from these in its action on some of the carbohydrates and glucosides and was not isolated from a scarlet fever patient.

Morphology. A pleomorphic streptococcus, showing irregularities in size and shape of elements, every transition between coccus form and bacillus form. This includes wedge shape, spindle shapes, oval forms, club shapes, and tendency towards filaments in agar, gelatin and modified Loeffler's blood serum. In hanging drops there were conglomerate coherent masses.

Staining Reactions. Stained readily by the ordinary aniline stains, intensely Gram positive and non-acid-fast. Metachromatic granules were not observed.

Cultural Characteristics. Agar slant, 37° C., three types of colonies after twenty-four hours: (*a*) gray, granular, irregularly outlined colonies; (*b*) colonies quite similar but showing a confluent appearance; (*c*) colonies evidently younger and smaller, which have a fine frilling of chains around a more dense compact confluent center.

Gelatin, 20° C., non-liquefying.

Bouillon, 37° C., clear, coherent sediment in bottom of tube.

Litmus milk, 37° C., twenty-four hours, coagulated rapidly, usually with quite a solid curd, lower half of tube white, while upper half was pink.

Carbohydrates and glucosides. Dextrose, lactose, saccharose and raffinose acidified, while inulin, salicin and mannite were not attacked. Neutral red showed some reduction.

Russell's medium, acidified. When, instead of a central stab, a stab was made next to the glass, the growth assumed a typical skyrocket appearance, spreading out near the bottom of the tube. The lower part of the tube was decolorized.

Position According to Andrew's and Horder's Classification. In the following table a comparison of the action of this pleomorphic streptococcus with other streptococci is given according to their action on various carbohydrates, glucosides and neutral red.

	Milk	Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Coniferin	Mannite
Pleomorphic streptococcus	+	+*	+	+	+	−	−	.....	−
Strep. equinus	−	−	+	−	−	−	+	+	−
Strep. mitis	A	−	+	+	−	−	+	−	−
Strep. pyogenes	A	−	+	+	−	−	+	−	−
Strep. salivarius	+	+	+	+	+	−	−	−	−
Strep. anginosus	+	+	+	+	+	−	−	−	−
Strep. fecalis	+	+	+	+	−	−	+	+	+
Strep. pneumonia	A	−	+	+	+	+	−	−	−

NOTE.—In the above table in the column for milk (+) signifies acid with clotting, (A) acid without clotting, (−) no apparent action. For neutral red (+) signifies reduction, (+*) partial reduction, (−) no action. Otherwise (+) signifies acid to litmus, (−) no action or alkaline.

Winslow[9] seems to think that the *Streptococcus scarlatina* described by Gordon and the *Streptococcus conglomeratus* of Kurth were the same as *Streptococcus pyogenes*. He bases his statement upon the further studies of Gordon upon this group from the standpoint of their fermentation reactions. It will be observed that the pleomorphic streptococcus described in this paper would, according to Andrew's and Horder's classification, correspond to *Streptococcus anginosis*.

Since this organism was isolated the author has made slides and cultures from 365 cases of tonsillitis occurring among students and faculty of the University and from other cases in the city, from several cases of bronchitis and one case of hand infection. In addition, fifty cultures and smears were made from the throats of apparently normal individuals. Streptococci with large cells and with rod-like cells were found very commonly in the cases of tonsillitis and frequently in bronchitis. Only a very few were obtained from the normal throats. These were from individuals subject to attacks of tonsillitis as a rule. Five strains were obtained from cases of tonsillitis, two from cases of bronchitis, and one from infection on back of hand of a member of the faculty, all of which showed marked tendency toward persistent involution and produced more marked conglomerate masses in the bottom of broth tubes. They correspond very closely in morphology, cultural characteristics and tinctorial reactions with the organism described above. From four of these cases fresh material was obtained and isolations of club-shaped organisms and chains with enlarged cells were made by Barber's pipette method for single

cell isolation. The individual organisms were inoculated into the moisture of condensation of agar slants, and after several hours the water was permitted to cover the surface of the slant. In this way growth was obtained. The author was unable to get very large individual coccus forms to grow satisfactorily, although Dr. F. Hecker has privately reported success. The resulting cultures from the above isolations showed as marked pleomorphic variations as the other cultures. As a rule, successive transfers in dextrose broth would yield uniform chains in time, but involution forms reappeared when transfers were made to solid media, especially if partially desiccated media were used. Very frequently beautiful chains could be obtained from the deeper part of the stab in Russell's medium when the stab was made next to the glass, while involution forms predominated upon the surface of the slant.

The *Streptococcus pyogenes*, and in fact the rest of the streptococcus group, is usually described as chains of cocci of comparatively uniform size, but varying in length of chain, pathogenicity, action on litmus milk, various carbohydrates, glucosides and neutral red.

Much work has been done which suggests that perhaps there are not so many different streptococci as has been generally thought, but that one or at most a few organisms make up the group and that these are very plastic and subject to much variation. Heineman[10] and others have concluded that *Streptococcus pyogenes* and *Streptococcus lacticus* are perhaps one and the same. Rosenow[11] has reported remarkably induced mutations in streptococci. He has converted hemolytic streptococci from many sources, such as erysipelas, scarlet fever, puerperal fever, arthritis, tonsillitis, milk, etc., into *Streptococcus viridans, Streptococcus mucosus,* and typical pseudopneumococci; *Streptococcus viridans* into *Streptococcus mucosus. Streptococcus hemolyticus* and *Streptococcus rheumaticus; Streptococcus mucosus* into *Streptococcus viridans* and *Streptococcus hemolyticus; Streptococcus rheumaticus* into *Streptococcus viridans* and *pneumococci.* In this work he made use of pure cultures isolated by Barber's pipette method for single cell isolation. Billings and Rosenow[12] record quite a remarkable observation of bacterial mutation from a bacillus to a coccus form. They report colonies on dextrose agar showing only bacillus forms yielded in subculture; a staphlococcus in pure cultures; and forms of the bacillus, either pure or in a mixture, anerobically on the same media.

Dr. Victor C. Vaughn,[13] citing the collected studies of Eisenberg on "Mutation in Bacteria," gives a splendid brief discussion of the subject. In conclusion he says: "Evidence of mutation in bacteria might be multiplied many times. It is shown in changes of form, in capsule formation, in production of spores, in alteration of virulence, etc., but I think that I have collected enough data to conclusively show that in bacteria acquired characters are in part at least inheritable."

The tendency toward pleomorphism was quite persistent for the streptococci reported upon in this paper. After seven months cultivation there was a tendency toward uniform chain formation, but decided involution forms would occur. The variation in morphology of streptococci in liquid media, such as mannite broth, saccharose broth, etc., reported by Jean Broadhurst[14] was much more temporary but quite interesting. She says: "The morphology of the individual cell may be likewise affected. In media not utilized, some of the chains are usually of full or increased length, and there is usually a small proportion of swollen organisms (rounded and elliptical) either in short chains or interposed here and there in chains composed mainly of normal organisms. When the media are utilized, there is, besides this swelling, a distinct tendency to abnormal shapes . . . Often, however, more varied forms are seen—organisms which may be actually pear-shaped, club-shaped or obtusely diamond shaped. These changes are most marked in mannite, though they may occur in other media. . . . . A normal appearance is effected at once by transplanting to plain broth." Jean Broadhurst also made another interesting observation, which was that a strain of streptococcus producing no change in litmus milk acquired the ability of producing acid and of coagulating the milk by being put into a capsule and passed through the intestinal tract of a dog.

Repeated attempts have been made without success to control the pleomorphism of these organisms. In view of the observation that involution forms appeared quite frequently upon partly desiccated solid media, it was thought that perhaps differences in osmotic pressures might be responsible for the variations. Accordingly bouillon containing different concentrations of dextrose were tried, as were also different concentrations of NaCl, but the involution could not be controlled or increased in slightly involuting strains. Then culture media containing different concentrations of HCl and NaOH were tried, with unsatisfactory results. Since in the first organism isolated it was possible to get

uniform chain formation under partial anerobic conditions, it was decided to try increasing the oxygen tension slightly above atmospheric and see if this had any effect upon involution. This gave unsatisfactory results, as well as an experiment in which the CO_2 content of the atmosphere in contact with the culture media was varied. These negative results naturally do not mean that these involutions can not be controlled, as no doubt that time and patience or good fortune would yield positive results. I might say, however, that some of my work on another problem has suggested that perhaps the pleomorphism on the partially desiccated agar was due to the salts that crystallized out on the surface of the agar. It is hoped that this may be checked up more fully in the future.

Even if involution forms could be produced at will under one set of conditions, it would not necessarily follow that these were the conditions holding forth in the body under which pleomorphism occurred or persisted. The variation of the morphology of these organisms from typical streptococci have been termed involution forms, largely because (1) these organisms seem to prefer, as a rule, to grow and act as streptococci under artificial conditions of laboratory cultures; (2) the streptococci as a group is known to be very plastic, mutations occurring in many different ways; (3) similar though more transient variations in morphology have been observed to occur in pure cultures of streptococci; (4) since sufficient evidence is not apparent to change the classification given them by Kurth, Gordon and Klein.

The similarity to *B. diphtheria* noted by Gordon and emphasized by Newman is at times quite striking. Not enough strains of these organisms were studied to warrant a statement that none might contain metachromatic granules, since it is a well-known fact, as emphasized by Graham Smith,[15] that not all strains of virulent *B. diphtheria* have metachromatic granules. None were observed in the cultures studied. I know that frequently these pleomorphic streptococci may be mistaken for *B. diphtheria* when smear preparations only are examined. A bacteriologist experienced in diphtheria examinations would not make the mistakes, but when one realizes that many relatively inexperienced individuals make throat examinations, either for themselves or others, it is quite possible for errors to be made. These errors would be upon the positive side and might or might not be objectionable, depending upon the tolerance of the individual for the foreign protein, any benefit derived from it, the expense incident thereto,

and the suffering entailed. Four of the eight severe cases of tonsillitis were given 10,000 units of antitoxin and made a rapid recovery. Of the other four cases, two cleared up in time though not nearly so quickly, and two developed ear infections and responded to vaccine treatment. All were suspected diphtheria cases. It might be said, in the first place, that the number of cases is too few for the results to be of value; second, that in the four cases receiving antitoxin, they would have made a quick recovery anyway; third, that in these four there were foci of infection with *B. diphtheria* in the trachæ (nose and throat examinations were made); fourth, that the diphtheria antitoxin was beneficial because of a relationship between the diphtheria group and this group (these organisms were not toxin producers in vitro); fifth, the benefit resulted from nonspecific protein therapy as emphasized by Muller, Weichardt, Miller and others for many infections. If the marked improvement resulted from the injection of the antitoxin, I would rather incline to the last explanation, perhaps, until a more definite relationship is proven between *B. diphtheria* and the streptococci than I have found. It is, however, true that Denny, Corbett and others have described strains of *B. diphtheria* that they call streptococcal forms, and the pleomorphic nature of *B. diphtheria* is well known. From the standpoint of morphology *B. diphtheria* has been frequently reported as showing involution forms similar to these pleomorphic streptococci. G. S. Graham-Smith[16] describes the involution forms of *B. diphtheria* as follows: "After prolonged growth on a suitable medium, or more quickly on an unsuitable medium, diphtheria bacilli become considerably altered in shape. Their appearance becomes more irregular and very large forms are frequently encountered. Many become greatly swollen at the ends and develop enormous club-shaped masses which stain deeply, while the rest of the bacillus may stain badly or have irregular patches of deeply stained protoplasm in it. Others become pear-shaped or globular, while some retain their general shape but become thicker throughout. Others again show large globules at their ends, while the rest of the rod appears as a faintly stained line. Specimens which take up the stain very badly are common. Some bacilli may be represented by small round masses like cocci, or by a chain of such masses, when they look like streptococci. In fact, under such conditions every variety of shape and form and staining capacity may be met with." The cultural characteristics of *B. diphtheria* are well known. It is generally considered that the organism ferments dextrose but not

lactose. Graham-Smith,[17] however, reports numerous strains of virulent diphtheria bacilli which produced acid with coagulation of Hiss's serum water medium containing lactose. The diphtheria bacillus grown in dextrose broth gives a typical streptococcus effect, clear broth with flocculent precipitate settling down. Macroscopically the colonies on agar are quite similar to normal streptococcus colonies. Its action in neutral red is variable. It must be remembered that one of the strong objections to classification of any organisms according to fermentation reactions is because of the fluctuating variability that one frequently meets with. I did not think that the pleomorphic streptococci isolated were identical with *B. diphtheria*, but I was interested in the apparent and suggested relationship between the streptococcus group and the diphtheria group, as suggested by Gordon and others. I next decided to compare the several strains of pleomorphic streptococci with each other and with *B. diphtheria* and *Streptococcus pyogenes* by means of the complement fixation test. I wanted to include a known culture of *Streptococcus anginosis*, but was unable to obtain it at the time. This work was then conducted as follows:

ANTIGEN. Two kinds of antigen were employed. In both cases the organisms were grown in plain agar. For one of the antigens suspension of the growth in isotonic salt solution was used. For the other type of antigen the growth was teased off in distilled water and suspension allowed to autolyze and an equal volume of double-strength salt solution added.

HEMOLYTIC SYSTEM. Human red blood cells and the corresponding hemolytic amboceptor.

TECHNIQUE. The technique, barring of course the differences in antigens, was similar to Noguchi's modification of the Wassermann reaction. As a preliminary to this work all of the animals were checked up for normal complement-fixing antibodies with each of the above antigens, and all were negative.

Following vaccination with the respective strains of organisms, the results showed cross-fixation for all of the strains of the pleomorphic streptococci, but no fixation when any of these pleomorphic streptococci were used as antigen with anti-*Streptococci pyogenes* immune sera or *B. diphtheria* immune sera, nor was the converse true. Neither was there any cross-fixation between *B. diphtheria* and *Streptococcus pyogenes*, although these antigens gave 4 plus reactions with their respective immune sera. In other

words, no relationship was apparent or suggested between these pleomorphic streptococci and *B. diphtheria* and *Streptococcus pyogenes* as suggested by Gordon. It might well be that a relationship could exist and the complement fixation test be unreliable. Meyer,[19] Aronson,[20] Kerner,[21] while not working with complement fixation test, decided that immune sera gave variable results and were not consistent. Some recent work on the streptococcus group in press is quite desirable. The complement fixation test has not been a uniform and decided success as a means of differentiation, but nevertheless is of great value in many instances.

Since this paper was finished two papers by Mellon on the diphtheroid group of organisms have appeared. He reports upon a pleomorphic organism isolated from the lungs which shows all variations from bacillary form to a streptococcus form. In morphology and many cultural reactions his diphtheroid is similar to the pleomorphic streptococcus I have been working with. Both are pleomorphic, Gram-positive, nonmotile organisms giving a clear broth with heavy sediment, coagulate milk, produce acid in dextrose, lactose, sucrose. The diphtheroid organism produced acid in salicin and inulin, whereas the pleomorphic streptococcus I am reporting on failed to do so. Mellon states that in broth cultures at one stage of development there are numerous bipolar bacilli with large granules. From this I presume that metachromatic granules were observed. I did not observe these occurring in the pleomorphic streptococcus studied. Doctor Mellon has placed his organism among the diphtheroids because of immunological, cultural and morphological reasons; however, he says that "unless great care is used in handling the recently recovered bacillary form, it will almost immediately revert to the diplococcus form when transplanted even to solid media." His experimental work and mine, made in attempts to control the pleomorphism, give largely negative results.

I should say that we were both working with closely related if not identical organisms. In his papers he suggests the immunological relationship between his organism and the group of diphtheroids, of *B. diphtheria* and the streptococci. As a result of complement fixation work, I found no relationship between the pleomorphic organisms I was studying and *B. diphtheria* or *Streptococcus pyogenes.*

SUMMARY AND CONCLUSIONS.

1. That a pleomorphic organism quite similar to if not identical with *Streptococcus scarlatina* of Gordon and *Streptococcus conglomeratus* of Kurth was isolated by me from chronically enlarged cervical lymph glands of an individual having no history of scarlet fever. This organism showed all transitions between bacillary form and chains of diplococci.

2. That a vaccine was apparently beneficial, and that since tonsillectomy no recurrence has taken place. The tendency for recurrence after three months is another example of the transient immunity resulting from streptococcus vaccination. It is a well-known fact that streptococcus infections do not confer a lasting immunity. This is one thing that should give us pause in ascribing to streptococci the primary cause of such diseases as scarlet fever and measles where a comparatively lasting immunity results.

3. That a similar organism was isolated from a hand infection, from two cases of bronchitis, and from five out of 365 cases of tonsillitis. Frequently pleomorphic streptococci showing other colony types on agar and Loeffler's blood serum were also observed in tonsillitis cases.

4. That some of the involution forms of this streptococcus might be mistaken for *B. diphtheria* by other than a well-trained observer. This is of importance in view of the many relatively inexperienced individuals doing laboratory work either for themselves or as a commercial proposition. It is of added interest in view of the observations of Gordon and Newman, suggesting a relationship between the streptococcus group and the diphtheria group. This has more recently been emphasized by Mellon.

5. That during the diphtheria epidemic in Kansas City in 1914 a number of patients showing only pleomorphic streptococci in nose and throat smears and cultures, nevertheless recovered rapidly following administration of 10,000 units of antitoxin. Similar results were obtained in a small series of cases in Lawrence. This may or may not mean anything. If the apparent beneficial results were real and not a coincidence, and if no unfound foci of diphtheria existed, it might not indicate a relationship between these organisms and *B. diphtheria*, but could be another example of nonspecific protein therapy as reported by Muller, Miller, Weichardt and others.

6. That according to Andrew and Horder's classification the pleomorphic organism I am reporting upon appeared to be the same as *Streptococcus anginosus*.

7. It ultimately seemed to prefer the streptococcus (chains of diplococci) morphology.

8. Complement fixation tests showed no normal complement fixing bodies, in the normal rabbits used, for any of the pleomorphic streptococci, *Streptococcus pyogenes* or *B. diphtheria*.

9. Complement fixation with respective immune sera showed no relationship between the pleomorphic streptococcus and *B. diphtheria*. This, however, does not necessarily prove no relationship.

10. While attempts to control the pleomorphism were largely negative, I have some results which suggest that the salts which crystallized out on the agar might be a factor in producing involution forms on the desiccated agar.

11. I feel that a healthy skepticism is well worth while in all work involving mutation, since errors in technique can lead to very erroneous conclusions. The work of Mellon seems to be thoroughly and carefully done and is worthy of consideration. His results, even more than mine, point to an intermediate group between the streptococci and diphtheria.

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CONTENTS:

THE FUNCTION OF THE SUPRAGLENOID CANAL, ... *Herman Douthitt.*

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The Function of the Supraglenoid Canal.

BY HERMAN DOUTHITT.

SEVERAL months ago the writer was discussing with Professor Williston the presence in *Diplocaulus* of the supraglenoid foramen, a structure which has been recognized in nearly all Permian reptiles, and in the Temnospondyli. Professor Williston stated that he had been surprised to note that the same foramen occurs in the lizards, where its presence had apparently been completely overlooked. He suggested that the writer study its function in the lizard, with the view likewise of determining its function in the early Tetrapoda.

With this end in view, the writer has made a careful study of the collared lizard, *Crotaphytus collaris*. Part of the material used was secured from the Dyche Museum of Natural History. I wish here to acknowledge the courtesy of Mr. C. D. Bunker in placing this material at my disposal. Fresh material has likewise been collected, and the arteries injected.

The supraglenoid canal, or foramen, occurs in two distinct forms. In lizards and Temnospondyli, in most Cotylosauria at least, and in some Thermorpha, as *Edaphosaurus* and *Ophiacodon*, its external opening is on the posterior edge of the scapula, at the bottom of the supraglenoid fossa. In the lizard, and no doubt in the other forms, the foramen lies just laterad of the area of insertion of the long head of the triceps muscle. In the lizard, however, there is no fossa. The canal passes from the foramen upward and forward, to open finally upon the inner surface of the scapula. In *Crotaphytus* and *Varanus* this canal is of considerable length, the inner opening being well above the middle of the scapula.

In most Theromorpha, and in *Diplocaulus*, on the other hand, there is no canal, and the foramen is not on the posterior margin, but farther forward, on the flat surface of the scapula.

It would seem that the form and position occurring in the lizards is the primitive one, since it is the type of both the Temnospondyli and the Cotylosauria. Apparently, the lizard is the only mesozoic or modern tetrapod which has retained this structure. Gunther shows a foramen piercing the scapula of *Sphenodon* (article not available to the author), but two scapulæ at hand show no evidence of it.

In determining the function of this foramen the writer has dissected four specimens of *Crotaphytus collaris*. It was seen at once that the foramen was used for the passage of a blood vessel, and was not used by the nervous system. In order to determine the exact relation and identity of this vessel, the

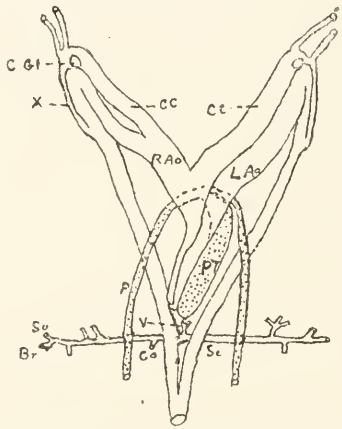

FIG. 1. Aortic arches and subclavian of *Crotaphytus collaris*. *Br*, brachiel artery; *CC*, common carotid artery; *C GI*, carotid gland; *LAo*, left aorta; *P*, pulmonary artery; *PT*, Pulmonary trunk; *RAo*, right aorta; *Sc*, subclavian artery; *Su*, subscapularis artery; *V*, vertebral trunk; *X*, Carotid-systemic connection..

subclavian and axillary arteries were traced out in detail. This very interesting system in the lizard has been traced out in detail for *Psammosaurus griseus* by Corti (1853). The writer does not have access to his account. Hoffmann's account in Bronn's Thierreichs is based upon Corti, but is necessarily condensed. It is not possible, therefore, to compare *Crotaphytus* with his account. Hoffmann makes no mention of the supraglenoid canal, from which it seems that it was not observed by Corti. Possibly the canal is not present in *Psammosaurus*.

In *Crotaphytus* (Fig. 1) the two subclavians come off from near the lower end of the right aorta by a single stem. After about 1 mm. this common stem divides, and the two subclaviæ pass directly laterad to the arm. At the point of their separation, in the median line of the body, the vertebral artery is given off as a single vessel. It runs directly forward on the ventral surface of the centrum for about 2 mm. and then divides into right and left vertebral arteries.

The costocervical axis leaves the subclavian just laterad of the centrum, and sinks at once into the muscles of the back.

Beyond the costocervical, the subclavian passes beneath the heavy *longus colli* muscle. Beneath the outer portion of this muscle it gives off two or three small branches which pass to the adjacent anterior regions. These should represent the thyrocervical axis. A little further laterad another vessel leaving the subclavian on the posterior side is no doubt the internal mammary artery.

The axillary artery divides almost immediately. The brachial continues down the arm in the usual manner. The lateral branch, which is without doubt the subscapularis, passes towards the space between the *latissimus dorsi* and a muscle which corresponds in position and relations to the *teres*. The subscapularis divides almost at once. The most posterior branch divides soon into three branches, which spread over the surface of the last-named muscle. The other branch turns towards the axis of the limb, over the proximal portion of the long head of the triceps, and into the space between this muscle and the humerus. Here it divides into several branches, one of which passes through the canal in question, to supply the subscapularis muscle.

There can be no reasonable doubt but that the supraglenoid foramen and canal in all forms in which it occurs, has the same function as the lizard; that is, that it serves as a passage for a branch of the subscapularis muscle. In mammals the subscapularis artery proper passes laterad of the scapula and its muscles, while only a minor branch passes to the subscapularis muscle. In the lizard, however, this branch seems to be the most important one.

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CONTENTS:

CHROMOSOMES OF NOMOTETTIX . . . . . *Myrtle F. Rayburn.*

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The Chromosomes of *Nomotettix*.

BY MYRTLE FRANCES RAYBURN,

University of Kansas.

THE Tettigidæ are a small, clearly defined group of grasshoppers known as the "grouse locusts." *Nomotettix* is one of the twenty-one genera comprising the members of this family found in North America. Of these, the cromosomes of representative species from four different genera, *Choriphyllum, Acridium, Paratettix* and *Tettigidea,* have been studied by Robertson ('15 and '16) and Harman ('15). *Nomotettix* will be the fifth genus to be added to this series.

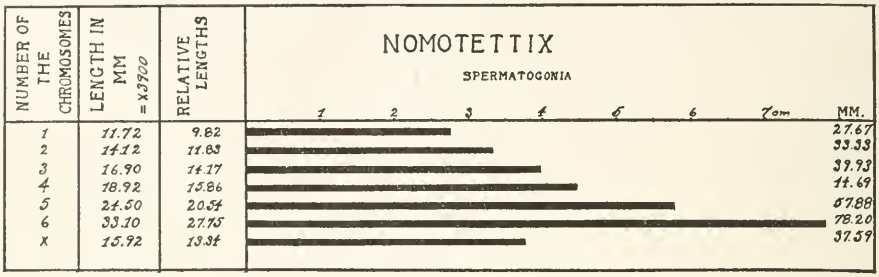
In his comparative study of the chromosomes of Tettigidæ, Robertson found a uniformity of numbers, and to a great extent of size relations throughout nine species belonging to the four genera above mentioned. In the species of *Nomotettix, cristatus* Scudder, which I have here studied, I find the same chromosome numbers and practically the same lengths of chromosomes.

The individuals used were from material collected near Lowell, Mass. The testes were fixed in Flemming's fluid and stained in Haidenhain's iron-hemotoxylin. Drawings were made with a camera lucida, and are here reproduced at a magnification of 2600 diameters.

The chromosomes of *Nomotettix* are of the rod-shaped type and are thirteen in number in the spermatogonia of the male. They may be readily arranged in a series of six pairs besides the accessory. There are two large pairs (Nos. 6 and 5), two intermediate pairs (4 and 3), and two smaller pairs (2 and 1). Usually the sex chromosome shows a woolly appearance, making it easy to distinguish. Its size is third in the series, coming between the 2's and 3's (autosomes).

CHROMOSOMES OF NOMOTETTIX.

Myrtle F. Rayburn.



In the table for *Nomotettix cristatus* Scudder, the first six horizontal bars represent the relative lengths of the six pairs of autosomes, and the seventh the percentage that the sex chromosome is of the total sum of the lengths of the autosomes.

In the relative lengths of chromosomes, the genus *Nomotettix* is more nearly like the genus Acridium than *Paratettix* or *Tettigidea,* as shown in particular by the measurements for the fifth and sixth pairs of chromosomes. Compare the relative lengths of these, 20.54 to 27.75, for the spermatogonia of *Nomotettix,* with those for the same chromosomes in *Acridium,* the genus to which *Nomotettix* is most nearly related taxonomically. Again, compare the relative lengths of the smallest and intermediate pairs with those of *Acridium.* It will be seen that there is not so very much difference. The smallest pairs, whose lengths are 9.82 and 11.83, compare very similar to the lengths 10.48 and 11.49 for *Acridium granelatus* or 11 and 11.06 for *A. incurvatus.* The intermediate pairs, 14.17 and 15.86, are slightly larger than in *Acridium,* the species *ornatus* excepted. These numbers in *Acridium* are as follows: For *granulatus,* 13.31 and 14.08; for *incurvatus,* 13.4 and 14.3; for *obscurus,* 13.3 and 14.1; but for *ornatus* 14.63 and 15.86.

The difference in length between the longer of the intermediate pairs and the shorter of the two longest pairs is about one-half as great as in the species of *Acridium.* Compare the ratio 15.86 to 20.54 of *Nomotettix* with 14.08 to 22.48 of *A. granulatus,* 14.3 to 22.5 of *A. incurvatus,* 15.86 to 21.47 of *A. ornatus* and 14.1 to 22.1 of *A. obscurus.* This is one point at which there is a rather marked disagreement between *Nomotettix* and *Acridium.*

In comparing the relative lengths of these chromosomes, as shown by the tables of my text figure, with those of Robertson for *Paratettix* and *Acridium,* it may at once be seen that

Nomotettix is farther away from *Paratettix* than from *Acridium*. For instance, there is a much less extreme range of length shown in *Paratettix* than in *Nomotettix*. Compare the ratios 9.82 to 27.75, the relative lengths of Nos. 1 and 6 of *Nomotettix*, with 11.4 to 23.6 and 11.4 to 23.7 of *Paratettix cucculatus* and *texana*, respectively, and also with 10.48 to 28.14 and 10 to 29.4 of *Acridium granulatus* and *obscurus*, respectively.

Likewise, the sex chromosome in *Nomotettix* is relatively shorter than in the species of *Paratettix*. Here it ranks between the second and third autosomes in spermatogonia, having the value of 13.34. In the spermatogonia of *Paratettix* it ranks between the second and third autosomes, but has the value of 13.9. In the first spermatocytes its rank is between the fourth and fifth autosomes and has a value of 17.2 in *Paratettix cucculatus* and 17.8 in P. *texana*. In *Acridium* it ranks either between the second and third autosome or below the first autosome. Its length is very short, having the value of either 11.95 or 8.7 in spermatogonia, or for first spermatocyte 13.45 (ranking between second and third, but low), 11 (ranking with No. 1), 7.9 (ranking below No. 1), and 12.09 (ranking between 2 and 3). It will be seen that in so far as size of the sex chromosome is concerned, *Nomotettix* is nearer to *Acridium* than to *Paratettix*.

As regards relations with the genus *Tettigidea*, *Nomotettix* is about the same distance away as *Acridium*. In this genus the two long chromosomes are relatively not so long, 22.3 and 24.6, and are more nearly equal in length, while in *Nomotettix* they are very unequal in length, as in Acridium. The sex chromosome in *Tettigidea* ranks fifth in the series, while in *Nomotettix*, as already mentioned, it ranks third.

CONCLUSIONS.

1. *Nomotettix* agrees with all other genera of the family Tettigidæ in having in the male six pairs of autosomes and one sex chromosome.

2. It agrees with those genera of the family so far examined in that the pairs of autosomes readily arrange themselves according to length into three groups—two shorts, two intermediates, and two longs.

3. In the relative lengths of the autosomes this species of *Nomotettix* agrees more readly with species of *Acridium* than

with species of *Paratettix*. This is to be expected, since externally the body characters of *Nomotettix* are more nearly like those of *Acridium* than of *Paratettix*. Likewise, this species of *Nomotettix* is much farther away from those of *Tettigidea* than from those of either *Acridium* or *Paratettix*. Again, this is to be expected from the fact that *Tettigidea* belongs to an entirely different subfamily, the Batrachidinæ. *Nomotettix*, *Acridium* and *Paratettix* belong to the subfamily Tettiginæ.

4. The sex chromosome may be recognized in spermatogonial divisions by its woolly appearance.

5. The chromosomes of *Nomotettix*, when compared as to numbers, relative sizes, etc., with those of other genera of Tettigidæ, furnish evidence decidedly in favor of the hypothesis that chromosomes are individual self-perpetuating elements.

This work was done under the direction of W. R. B. Robertson.

JUNE 1, 1917.

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EXPLANATION OF PLATE.

Magnification at which drawings were made, × 3900. Reproduced here at × 2600. Small arabic numerals indicate the autosomes in the order of their size, from smallest to largest. In spermatogonia there are two of each size. The sex chromosome is indicated by an *x*. The numeral preceding the *x* indicates that the sex chromosome in size occurs between the second and third autosomes.

PLATE I.

Nomotettix cristatus Scudder.

FIGS. 1 TO 7. Spermatogonia dividing, metaphase stage.

CHROMOSOMES OF NOMOTETTIX.
Myrtle F. Rayburn.

PLATE I.

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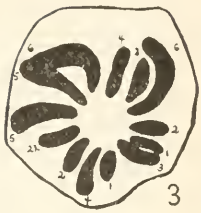
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THE

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CONTENTS:

CHROMOSOME STUDIES IV: A DEFICIENT SUPERNUMERARY ACCESSORY CHROMOSOME IN A MALE OF TETTIGIDEA PARVIPENNIS,
W. Rees Brebner Robertson.

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Chromosome Studies IV: A Deficient Supernumerary Accessory Chromosome in a Male of *Tettigidea parvipennis*.

W. REES BREBNER ROBERTSON,
University of Kansas.

THE normal number of chromosomes of the species of *Tettigidæ* ("grouse locusts") so far examined is thirteen and fourteen in the male and female, *i. e.*, twelve autosomes and either one or two X-chromosomes, respectively. (Robertson '16; Rayburn, '17.) The subject of this investigation is a specmen which possesses the secondary sexual characters of a male, yet has fourteen chromosomes. What makes the case still more striking is the fact that the extra chromosome is probably an X-chromosome. On measurement, however, it was found to be shorter than its mate by between one-fifth and one-fourth the normal length (figs. 15, 17, etc.). As will be seen, it is probably to be considered a supernumerary, but deficient, accessory chromosome.

Its occurrence in the individual is uniform for all cells found dividing, somatic or germinal. In somatic cells it is not so readily identified in every case yet the fact that the number fourteen may always be clearly counted indicates its presence when other means fail. The somatic cells found containing it were from "fat body," follicle wall, and *vasa efferentia* tissues. The chromosomes in these cells are large and widely separated. The drawings indicate their actual positions in most cases (figs. 1 to 5). No difficulty or doubt was had in making the count here. Of course, counts in somatic cells could be obtained only from tissues surrounding the testes,

since these were the only tissues that had been taken with the testes from the body of the animal at the time of cytological preservation. The carcass itself unfortunately was preserved in a weak solution of formalin. The fact however, that "fat body" cells and the cells of the testicular follicles and of the *vasa efferentia* show fourteen chromosomes is reason for thinking that this number would likely have been found in all somatic cells.

In the germ cells (spermatogonia, figs. 6 and 7; first spermatocytes, figs 8 to 21; and second spermatocytes, figs 22 to 27) this chromosome is uniformly present. It is here that, on account of the singular behavior of accessory chromosomes normally exhibited in spermatogenesis stages, a clew is first obtained as to the probable nature of this supernumerary body which resembles in every way an accessory chromosome. Its characteristics are as follows:

(1) It may be recognized along with the accessory in synapsis and growth stages by its condensed condition (figs. 8 to 16).

(2) In "bouquet" of synapsis, intermediate finely threaded or netlike, and early prophase stages of the first spermatocyte, it always lies near the wall of the nucleus, which is characteristic of the accessory chromosomes (figs. 8 to 13).

(3) It condenses ahead of the other chromosomes in prophases of the first spermatocyte (figs. 14 to 16).

(4) It has a tendency to pair side to side with the accessory during parasynapsis stages when other chromosomes are similarly pairing (figs. 8 to 11). This occurs in about from 65 per cent to 75 per cent of cells. At this time both the accessory and the supernumerary have the form of a right-handed corkscrew like spiral (fig. 8). At later stages, they lie not so closely together, though there is evidence, in many of the cells, that they are in some way attached to each other (figs. 14 to 19). The synapsis seems to be most intimate at the pointed ends, which, judging from the general position in the cell and from the direction of orientation of the ordinary chromosomes, I have a surmise may be the distal ends. Further evidence of the association or pairing which occurs in synapsis is afforded by their behavior in the first spermatocyte division (figs. 17, 18, 19). In some cases (fig. 17) they are traveling to the same pole closely approximated to each other; in other

cases (figs. 18, 19) they are traveling to opposite poles, but separating from each other much like the members of autosome pairs that have been in synapsis.

(5) When the conjugated pairs of chromosomes (tetrads) arrange themselves in the metaphase of the first spermatocyte division, the supernumerary body passes like an accessory, undivided to one pole, going either to the same pole as the accessory chromosome (figs. 17, 21), or to the opposite pole (figs. 18, 19, 20).

(6) The supernumerary appears in one-half of the second spermatocytes either with the accessory or without it (figs. 22 to 27).

(7) It divides in this division as does the accessory chromosome (figs. 26, 27). One-half the spermatids get it and one-half do not. Four sorts of spermatozoa accordingly are formed.

From the characteristics here given, it is evident that this body is probably an accessory chromosome. If it occurred in these cells in the absence of the normal accessory (No. 5*x*) it certainly would be taken for that body. It fills all the specifications for the accessory of *Tettigidea* except size. An idea of the difference in size may be gotten by comparing the lengths of the two chromosomes in figures 8, and 15 to 21. The proximal end, from which the spindle fiber springs, seems to be normal, but the distal end appears somewhat abnormal (figs. 14*a*, 15, 17, 18). It seems likely that this chromosome is a descendant of an X-chromosome which at some previous polar body formation failed to separate properly from its mate, losing thereby a portion from its distal end. An incomplete daughter chromosome such as this might result. In this connection it may be said that it behaves very much like the deficient No. 4 chromosome described in Studies III (Robertson, 1915), whose peculiar structure and behavior was accounted for in this way.

In the literature on supernumeraries, the nearest approach to this chromosome are the cases described by Stevens in *Diabrotica soror* and *D. 12-punctata* (1908 *b*, 1912 *a*) and in *Ceuthophilus* (1912 *b*). Normally in these species there is a large unpaired heterochromosome (accessory) in the male and two such in the female similar to what occurs in Orthoptera. Stevens found about 50 per cent of the males of the

Diabroticas to have from one to five supernumeraries and 20 per cent of the males in *Ceuthophilus* to have one or more supernumeraries. Her conclusion was that these bodies were probably related to X-chromosomes, descended from fragments of X-chromosomes that had arisen through irregularities in the maturation division at some previous time. Wilson regarded his supernumeraries in *Metapodius* as duplicates of the Y-chromosome, *i. e.*, of the smaller member of the idiochromosome pair. No such Y-chromosome has been described for Acrididæ or Locustidæ. In addition, the irregular behavior of this supernumerary body (*s*) with reference to the accessory chromosome (5*x*), in going either with it or not to one pole in the first spermatocyte, is evidence against its being a Y-chromosome. This was also true of the *Diabroticas* and *Ceuthophilus*. It differs, however, from the latter two cases in this: that no cell has been found showing this body dividing in first instead of the second spermatocyte division, as was true in a few cases in the *Diabroticas* and *Ceuthophilus*. Otherwise it behaves very much like the supernumeraries of these forms. It seems warrantable to consider it as belonging to the X-chromosome rather than the Y-chromosome category.

If this body is in reality an accessory chromosome, and one that has lost from the distal end one-fifth or one-fourth of its length, and if it is also to be considered functional in so far as the portion present is concerned, we may regard it as throwing some light upon the question as to how much and what portion of the accessory in the family *Tettigidæ* is concerned with sex-determination. In the first place, that the sex-determining genes are probably limited to only a portion of the accessory chromosome in the genus *Tettigidea*, at least is to be inferred from a comparison of the relative lengths of the chromosome in the different genera of the family to which the genus belongs, the chromosome varying from eight in *Acridium* to fourteen or fifteen in *Paratettix* and eighteen or nineteen in *Tettigidea*. This supposition is of course based upon the assumption, first of all, that the X-chromosome is to a large degree similar in constitution throughout the family. That this is true we have reason to believe from the fact that these genera which appear to be so closely related taxonomically possess chromosomes (both autosomes and allosomes) which have a very great degree of similarity in number, form, and relative size (Robert-

son, '16; Rayburn '17). Such could only arise if the chromosomes of the group are considered as genetically continuous and related. The same applies to the X-chromosome. Further evidence that sex chromosomes may be similary constituted throughout a series of related species is afforded by the work of Metz ('14, 16*a*, 16*b*, 16*c*, 16*d*) on the *Drosophilas*, which has shown that not only one plan of structure, in so far as relative sizes, etc., is concerned, may run through the chromosomes of each species of a genus, but, what is still more striking, that each gene has probably the same locus with reference to other genes along the length of the chromosome throughout the various species. If such be the case in the *Drosophilas* it seems a probability that the same might be true of the accessory chromosome among the genera and species of the *Tettigidæ*. Then, we are justified in surmising that, since in the genus *Acridium* the sex-determining chromosome has a value of 8, in the genus *Tettigidea*, where its length is relatively 19, only a portion of this chromosome, considerably less than half, *i. e.*, about eight nineteenths, could be concerned with sex-determination.

If a comparison of the sex chromosome in the genera of this family leads us to believe that the sex-determining portion in the genus *Tettigidea* is at most not greater than eight nineteenths of the total, in the case of the supernumerary accessory described in this "Study" we have evidence, it seems, which limits the size of this portion to a still greater degree, namely, between one fifth and one fourth the length of the chromosome. The reasons for concluding this are as follows: The presence of the chromosome in this particular animal has not produced a female; hence it must lack the part concerned with sex determination. Bridges ('16), in his splendid work upon nondisjunction of pairing sex chromosomes in *Drosophila amphilophila*, has shown that sex determination may be excluded from one portion of the X-chromosome at least, and therefore limited to a part of it. He found that a small portion of the X-chromosome in the region which carries the genes for "barring of the eye" and "forked condition of the bristles" became nonexistent genetically (pp. 150, 151). Females could exist with one such deficient X-chromosome. The sons of such heterozygous females were only one-half as numerous as the daughters, and did not possess the deficiency. The evidence warranted the conclusion that males could not exist with such

a deficient chromosome, Bridges' discovery has demonstrated, in the first place, that deficiencies may occur in X-chromosomes; in the second place, that the sex differentiators are limited to a part of the X-chromosome, for they did not occur in that portion as least of the chromosome which has to do with the above genes and which lies between the loci of the genes for "rudimentary wing" and "fused." Now if such be the case in *Drosophila,* then in *Tettigidea* we are justified in concluding that the sex determinants may likewise be limited to a portion of the chromosome, and since the animal which has here been described as possessing one normal and one short X is a male instead of a female, it seems safe to conclude that possibly the missing portion of this chromosome contains the determiners for sex, and further that in the genus *Tettigidea* at least these determiners lie near one end, probably the distal end, of the chromosome.

TIME OF APPEARANCE OF THE LONGITUDINAL SPLIT IN SOMATIC CHROMOSOMES

This discussion can not be closed without reference to the evidence afforded in figures 2 to 5 upon the question of the time at which division of the chromosomes in somatic mitoses takes place. In figures 2 and 3, late prophases of cells from the follicular wall, any portion of a chromosome which happens to run at all vertically shows distinctly in cross-section a secondary split at right angles to the very wide and apparent primary split. In figure 3, at the arrow point, such a portion of chromosome No. 4 is drawn. Most of the chromosomes here show the same. These cells have not yet entered upon the metaphase stage of division. Division, when it comes, will take place in the plane of the very apparent primary split. The secondary split here present can only be for the next again metaphase. This is shown much more conclusively in anaphases (figs. 4 and 5) from the same tissues. Here it is certain that division along the primary plane has taken place. The daughter chromosomes in traveling to the poles of the spindle show clearly the secondary split (fig. 5). It must be remembered that these chromosomes are entering upon the telophase stage of division, and go from that to the "resting" condition of the cell. Such a split is likely the forerunner of

the split which is most apparent in the prophase figures 2 and 3.

This is not the first time that such a phenomenon has been described. Dehorne (1911) found the same to occur in the somatic mitoses of *Salamandra*. In addition, I have reason to believe that this phenomenon occurs in the chromosomes of the first spermatocyte of the *Tettigidæ*, while they are yet in the daughter-cell stage resulting from the last spermatogonial division. There it seems that the chromosomes, before entering upon the stages of side-to-side pairing in synapsis, are in reality definitely split along a longitudinal plane. Pairing takes place evidently between members that are already made up of two strands, and of course the four-part tetrad results.

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EXPLANATION OF PLATES I, II, AND III.

Drawings were made by aid of a camera lucida and are here reproduced at 2600 diameters. The pairs of autosomes are numbered, according to size, from 1 to 6. The normal "accessory" chromosome is numbered *5x*. The numeral *5* indicates that it ranks between the fourth and fifth autosome in size. *S* designates the deficient supernumerary "accessory" (?) chromosome.

FIGS. 1 to 5. Divisions in somatic cells each showing 14 choromosomes.

FIG. 1. Fat body cell. No. 5's are foreshortened.

FIG. 2. Cell from the follicular wall of the testis. Each large cromosome shows at the proximal end a peculiar somewhat knoblike portion, which seems to be less deeply stained. The chromosomes are woolly. The longitudinal split marked.

FIG. 3. Cell from follicle wall. Follicle itself was full of spermatozoa. Chromosome No. 5 is foreshortened. Each choromosome in figures 2 and 3 is split twice longitudinally. This may be seen by focusing on parts of chromosomes running vertically. See cross-section of No. 4 at arrow point.

FIG. 4. One of the daughter cells, in a late anaphase or telophase of a follicular cell. Distal ends of these chromosomes show the secondary split. That which takes place at the division which follows the resting stage into which this cell is about to pass.

FIG. 5. Cross-sectional view of a daughter group of chromosomes in the anaphase stage similar to figure 5. Chromosomes very long. The secondary split is quite visible. This cell will soon pass into the resting stage. Cell taken from wall in region of the neck of the follicle.

FIG. 6. Spermatogonium in metaphase stage of division. Fourteen chromosomes.

FIG. 7. Similar spermatogonium from another follicle.

FIGS. 8 to 21. Stages of first spermatocytes from early synapsis to division.

FIG. 8. "Sheaf stage," corresponding possibly to the "bouquet" stage of other authors. The numbers of each of the six pairs of autosomes are in parasynapsis at one end, probably distal, where six winding threads may be counted. The normal accessory chromosome (*5x*) and its deficient

mate (*s*) lie to one side of the nucleus coiled in corkscrew fashion, the spiral turning toward the right. Both point to what is probably the distal end of the cell.

FIGS. 9*a*, 9*b*. No. *5x* and *s* pairing in synapsis stages slightly later than figure 8.

FIGS. 10*a*, 10*b*. Similar stages. Each accessory shows a longitudinal split.

FIGS. 11*a*, 11*b*, 11*c*. Similar stages; all taken from one cyst. Accessory chromosomes usually lie near together, but may lie on opposite sides of the nucleus. Inequality of the two is apparent.

FIGS. 12, 13. Fine spireme stages following the bouquet stage. The accessory and its supernumerary mate may lie close together or on opposite sides of the nucleus. Both always lie on the nuclear membrane.

FIGS. 14*a* and 14*b*. Cell from two sections. Early prophase. Six pairs of autosomes form tetrads. The normal *5x* accessory and its mate near by. Note shortness of the supernumerary. Both are split.

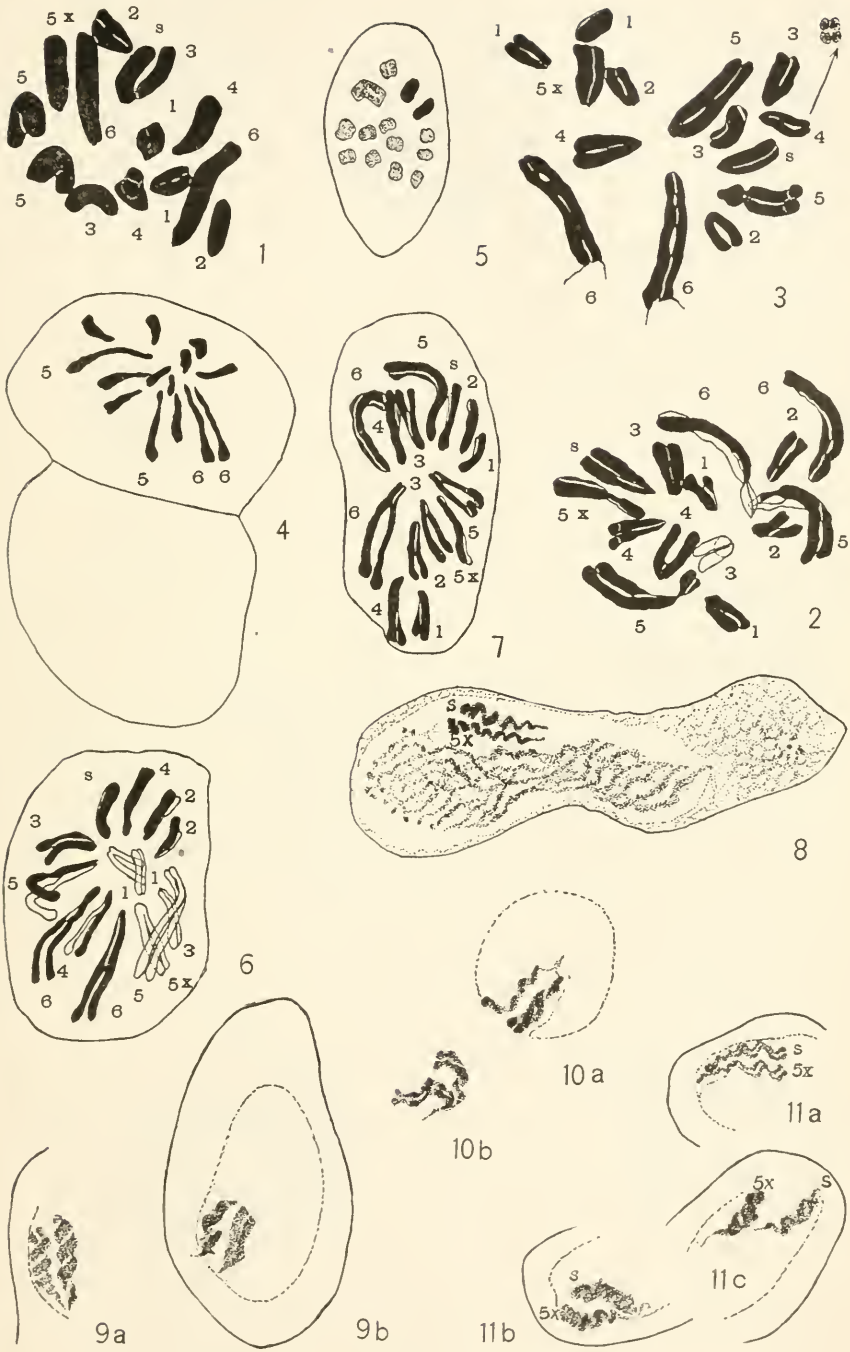
FIGS. 15*a*, 15*b*, 15*c*. Three similar stages from other cells of the cyst. Both chromosomes split.

FIG. 16. Similar stage; complete cell. Chromosome No. 6 moved outward.

FIGS. 17 to 21. Division of first spermatocyte. Supernumerary going either with the accessory or to the opposite pole. In 65 to 75 per cent of the cases it lay near the accessory, but might not (figs. 20, 21). All other chromosomes of normal size and dividing normally. Chromosome No. 5 slightly displaced to right in figure 17.

FIGS. 22 to 25. Second spermatocyte division, polar view, showing six, seven and eight chromosomes, depending upon whether No. *5x* and *s* were absent or present, either one at a time or both.

FIGS. 26, 27. Second spermatocyte division, lateral views of the spindle, showing that all chromosomes divide. Six chromosomes in one, the *5x* and the *s* chromosome absent; eight in the other, both the *5x* and the *s* chromosome present.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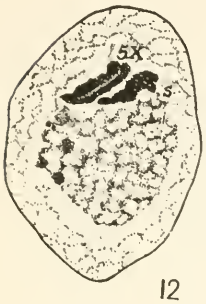

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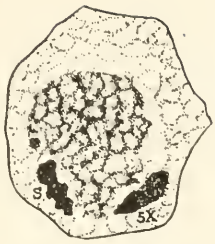

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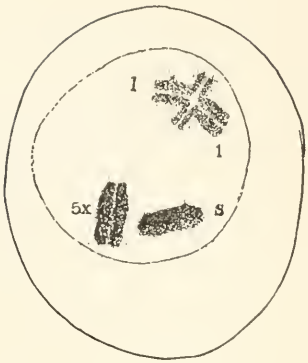

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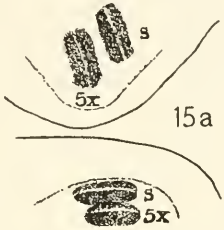

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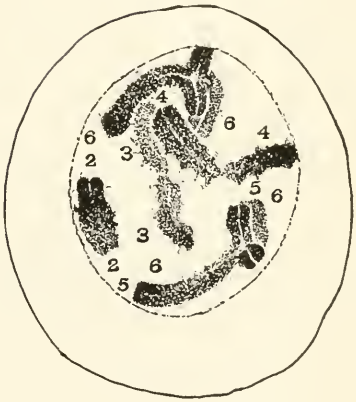

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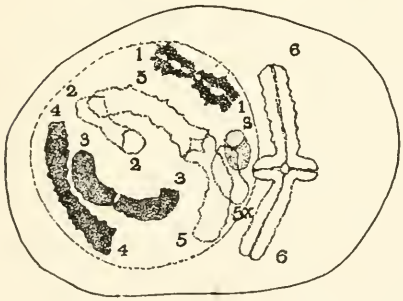

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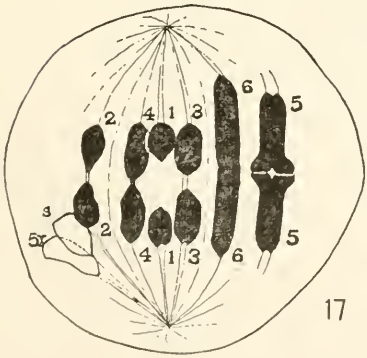

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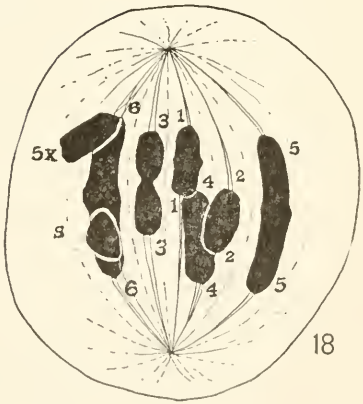

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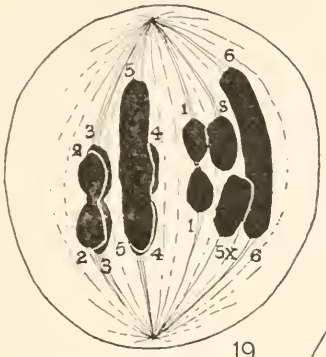
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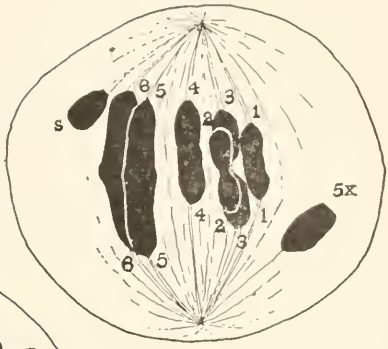
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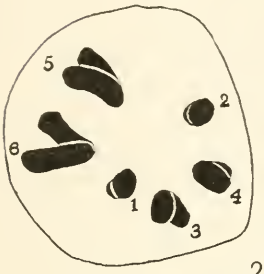
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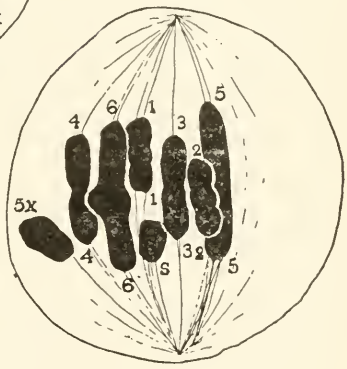
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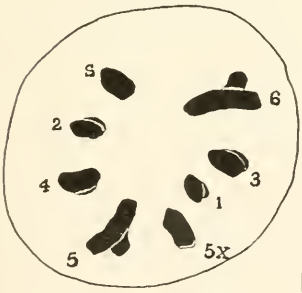
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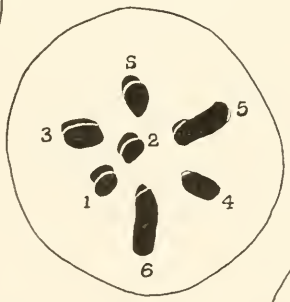
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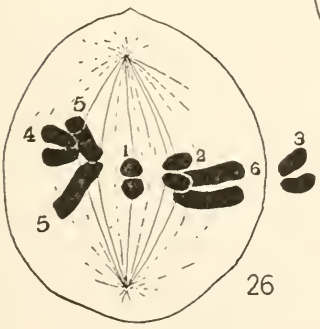
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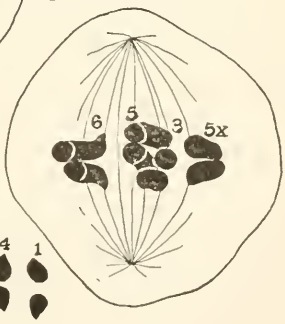
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A Mule and a Horse as Twins, and the Inheritance of Twinning.

W. REES BREBNER ROBERTSON,
University of Kansas.

THIS occurred on the farm of George E. Loy, of Barnard, Kan., June 9, 1913. On hearing of the case, in March, 1916, I looked it up and found, from the testimony of Mr. Loy and his family and neighbors, and that of the photographers who took the pictures and from my own observation, that the following are the facts concerned.

In 1912, the dam (III_9), just ten minutes before being bred to the ass stallion (III_7), had been bred to a young three-year-old horse stallion (III_6), which was being used as a part of the technique sometimes thought to be necessary in securing the fecundation of the female horse by the male ass. In such cases fertilization is expected from the second sire. In this instance, however, there resulted twins, one of which was a male horse, the other a female mule.

The case in itself was of sufficient interest, fortunately, to be photographed at the time by the local photographer, who sold the pictures (fig. 1) at fifteen cents apiece. Photographs were also taken later by the district school teacher (figs. 2, 3 and 5). Photographs 4 and 6 were taken by myself. Unfortunately, the mare (III_9) had died of old age during the wintertime preceding my visit, and the horse foal (IV_{10}) had died of exposure and poor feed the winter of 1914-'15 at eighteen months of age. But in spite of these discouragements, the data given by Mr. Loy and his children and by his neighbors checked up in every way so well with the evidence afforded by the photographers, and with what I have been able to observe myself, that it seems worth while to put the case on record.

On investigating the history of the mare I found that in the nine times she had produced foals she had given birth to twins (mules) on two other occasions, the fifth and ninth times (see plate I). In addition, her second foal (IV_3) had given birth to twins (horses), and also her own half sister (III_{10}) had produced twins, though the latter came too soon, being "slipped" at nine months gestation. Evidently there must have been present in the family the trait of twinning handed down from mother to daughter, and through three generations, starting with the Oregon "herd" mare.

That this twinning is of the dizygotic sort is proven by the differing colors and markings in all of the cases, and in two cases by differing sexes. In the first pair (IV_6 and IV_7) both were males; but one was black, the other brown. The black one lived only two days. In the second case occurred the mule and horse pair (IV_{10} and IV_{11}), which certainly must have been dizygotic. They differed in sex and in marking, though both were bay, but one a light bay (mule), the other darker bay (horse). In marking, the mule had a small crescent-like white blaze to the right of the center of the forehead (fig. 4). The horse was of uniform bay color with black mane, tail and feet.

In the third case (IV_{12} and IV_{13}) the twins were mules of different sex and marked differently, the female (IV_{13}, figs. 5 and 6) having a white spot in the forehead and in general color darker, with less of the light tan color characteristic of mules about the muzzle and under-barrel parts, while the male had no white markings, was generally lighter in color, and had, as the photos will show, more of the light tan color about the muzzle and ventral parts.

In the fourth case (IV_{14} and IV_{15}), in which twins were produced by a half sister (III_{10}) of the mare III_9, the foals were aborted at between 8 and 9 months gestation. The sexes, colors, etc., were not recorded.

The fifth case (V_1 and V_2 was a pair of horse twins, both of the same sex but differing in color and markings, from the daughter (IV_3) of III_9. They did not live to maturity. One was a solid bay like the mother IV_3 and her sire III_1, while the other was sorrel with a "bald" white face and white feet, similar to the grand dam III_9 and the great grand dam (Oregon mare) II_4, and also to IV_1 (figs. 3 and 4), a full brother to the dam IV_3.

As the pedigree (plate I) will show, the mare was heterozygous for the presence of the bay factor, since her sire was black and her dam had the bay variation of chestnut, known (Castle's "Genetics and Eugenics") as sorrel. This probably gave her the ability to throw the black and brown coats of her first twin mules (IV_6 and IV_7) and also probably the darker and lighter brown coats of her third twin mules (IV_{12} and IV_{13}). That she carried the bay factor is certain from the fact that by a black horse stallion (III_6) she produced a solid bay colt (IV_{10}). Her daughter was probably heterozygous for black, which gave her, when bred to a sorrel sire (IV_2), the ability to throw in her twins one a sorrel, the other a bay like herself. This daughter must likewise have been heterozygous for spotting, *i. e.*, assuming spotting recessive. She herself was solid bay, but her mother (III_9) had white face and white points and one of her twin colts was similarly marked. These foals did not live to maturity.

By way of summary, these facts, then, show that in all probability each case of twinning in this family is one of the dizygotic type, due to the simultaneous, or nearly so, fertilization of two distinctly separate unlike ova by two unlike spermatoza. Of the four cases where observations were made, this is shown in two by differing sexes and at the same time differing general coat color and widely differing markings; in the two remaining cases by differing general coat color, black and sorrel or black and brown, and at the same time differing spotting with white. In addition, one of the former cases, where the sex of the pair is different, one foal is a mule and the other a horse.

A second conclusion is that in these cases we have evidence of the inheritance of the tendency to produce heterologous twins through three generations, transmitted evidently from the bald-faced sorrel Oregon mare (II_4), since two of her daughters (III_9 and III_{10}), by different sires, possessed the trait.

Likewise, it is probably to be concluded that in order to be the producer of such twins, the dam herself need not have been a twin, since neither III_9, III_{10} nor IV_3, all of which produced twins, were themselves twins. It must not be forgotten, however, that possibly the twin in each of these may have been formed but failed to develop.

In the case of the mule and horse twins, it might be supposed that the presence of a mule fœtus might in some way affect the development of a horse fœtus, and accordingly some abnormal characters appear in the horse (Lillie, '17). So far as I can see, no such characters are present. From the photographs that were taken (figs. 1-3), one can not detect mule characters. In figure 3 the hair is long, due likely to winter conditions. The photos of the mule show it likewise to be a normal animal.

The present occurrence of a mule and a horse as twins is probably what might be termed a case of superfecundation rather than superfœtation, since fertilization of both eggs could have taken place only at one period of heat. We have reason to think this from the fact that only one breeding to each sire occurred, and these breedings at a ten-minute interval. Mumford, in his text "The Breeding of Animals," gives seven examples of what he terms superfœtation. Six of these resulted in twin colts, one of which was a mule, the other a horse; but they resulted from fecundation at a second or later period of heat. One of the colts, accordingly, was usually smaller or less well developed than the other. In two of these cases both died at birth or soon after; in one case he does not say; in two cases one twin only died; while in one case (his plate II) both evidently lived. From the number of cases cited by Mumford, it may be seen that the occurrence of mule and horse twins is not such a rare phenomenon as one might expect. The facts in the case here presented and in the other cases probably indicate that such twins may be produced, not only by fertilization during pregnancy at a recurring period of heat, as Mumford's cases seem to be, but also as a result of the appearance of two ova in the uterus at once.

Newman, in his book "The Biology of Twins," p. 95, says: "At the present time, I have no reliable evidence of twinning in horses, but it is highly probable that this group offers no exception to the general rule that all mammals normally producing but a single offspring at a birth may have twins." The present case and those of Mumford, I believe, are now good evidence of twinning in the horse, and twinning in the case here described is shown to be of a hereditary nature.

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EXPLANATION OF PLATES.

PLATE I.

I_1. Black percheron stallion.
I_2. Black mare.
I_3. Full blood Arabian stallion; spotted over hips.
I_4. Wild pony mare from herd; color was black with large white spots.
II_1. McDuff, a black percheron stallion. Service charges, $25.
II_2. Black mare.
II_3. Arabian stallion; white, with some black spots.
II_4. Oregon mare, branded; color, sorrel; had white points from knees and hocks down, and a blaze in face.
III_1. Horse stallion; dark bay.
III_2. Ass stallion.
III_3. Ass stallion; black.
III_4. Ass stallion.
III_5. Ass stallion.
III_6. Horse stallion; black. Not related to III_9. Used to tease before breeding to ass stallion. Was two years old, coming three, at the time.
III_7. Ass stallion, "Mammoth" breed; gray. Owned by farmer W. T. Watson of Barnard, Kan. Used ten minutes after III_6.
III_8. Ass stallion.
III_9. Sorrel mare with white face and white points. (See figures 1, 2 and 3.) Face spot slightly to right. Foaled 1892. Mother of the mule twins, IV_6, IV_7; the horse-mule twins, IV_{10}, IV_{11}; and IV_{12}, IV_{13}. Died winter of 1915-'16.
III_{10}. Half sister to III_9, dam of twins IV_{14} and IV_{15}. Was owned by Mr. Loy's mother.
III_{11}. Horse stallion.
IV_1. Horse (gelding), sorrel. Foaled 1899. Markings and color (see fig. 4) same as mother III_9.
IV_2. Horse stallion; sorrel.
IV_3. Mare; solid bay. Foaled 1901. Mother of twins V_1 and V_2.
IV_4. Horse (gelding); solid bay. Foaled 1902.
IV_5. Mule (male).
IV_6, IV_7. Pair of male mule twins; one black, one brown. Black one died 48 hours after birth. "Its bowels did n't work. Veterinary gave it oil; then it scoured to death."

IV_8. Mule (female).

IV_9. Mule (female).

IV_{10}. Horse (male); bay with no spotting. (See figs. 1, 2 and 3.) Twin to mule IV_{11}. Normal. Lived into the second year, probably eighteen months. "Died of natural causes; poor feed and exposure in second winter (1914-'15."

IV_{11}. Mule (female). Twin to horse IV_{10}. Solid light bay; slight blaze to right of center of face. Normal strong mule. (See figs. 1, 2 and 4.)

IV_{12}. Mule (male); bay or brown. Twin to IV_{13}. No blaze on forehead; slightly lighter in color, and with lighter muzzle and under parts than its twin mate. (See figs. 5 and 6.)

IV_{13}. Mule (female); bay or brown. Twin to IV_{12}. Blaze on forehead; general color slightly darker, and muzzle and underparts slightly darker than twin mate. (See figs. 5 and 6.)

IV_{14}, IV_{15}. Pair of horse twins. Sex unrecorded; foaled too soon ("slipped") by half sister to III_9, at between eight and nine months of gestation.

V_1. Horse (male). Twin to V_2. Sorrel with bald face and white points like grand dam III_9. Did not live to maturity.

V_2. Horse (male). Twin to V_1. Solid bay. Did not live to maturity.

PLATES II, III AND IV.

FIG. 1. Photograph of mare III_9 and her horse-and-mule twins (IV_{10} and IV_{11}) at three days after birth, taken by a local photographer. Persons photographed are sons of the owner.

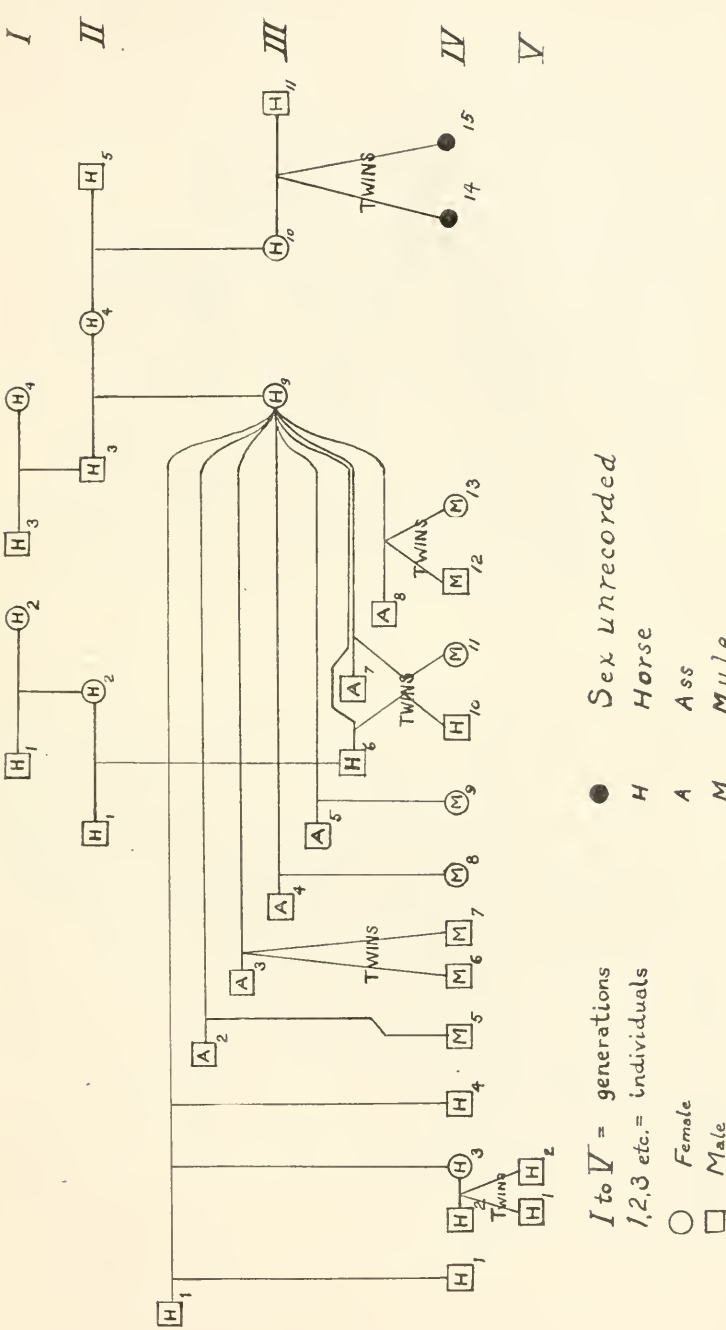
FIG. 2. Photograph of mare III_9 and her horse-and-mule twins, taken by the local school teacher in winter of 1914-'15.

FIG. 3. Photograph of mare III_9 and the horse colt twin, taken at same time by same party at closer range.

FIG. 4. The mule twin IV_{11}, in harness at thirty months of age. The horse IV_1 is the first colt of the mare III_9. Photo by the author, March, 1916.

FIG. 5. Third pair of twins (mules), IV_{12} and IV_{13}, at five days of age. Taken summer of 1915.

FIG. 6. Same as fig. 5, but lateral view. Photo by author, March, 1916.


I
II
III
IV
V
TWINS
TWINS
TWINS
TWINS
TWINS
I to V = generations
1,2,3 etc. = individuals
Female
Male
Sex unrecorded
Horse
Ass
Mule

III 9 & IV 10 & 11. Fig 1

III 9 & IV 10 & 11. Fig 2

III$_9$ IV$_{10}$ Fig 3

IV$_{11}$ IV$_1$ Fig 4

IV 12 & 13

Fig 5

IV 13

IV 12

Fig 6

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